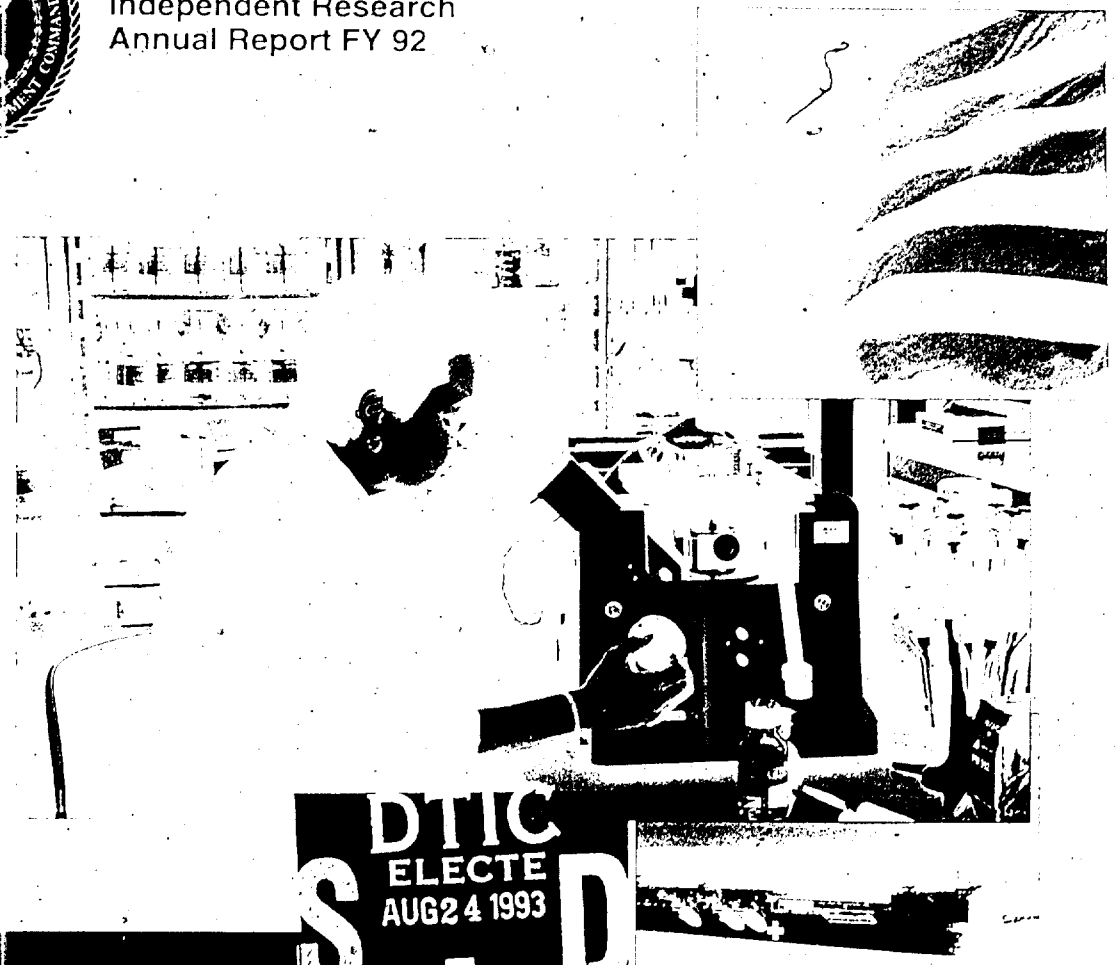
Independent Research Annual Report FY 92



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Work Unit Numbering

Work units are coded using the numbering series listed below to indicate the laboratory or detachment at which the research was performed.

Work Unit # Series	Laboratory/ Location
0000	Naval Dental Research Institute Great Lakes, IL
	Naval Dental Research Institute Detachment Bethesda, MD
	Naval Dental Research Institute Detachment San Antonio, TX
1000	Naval Medical Research Institute Bethesda, MD
	Naval Medical Research Institute Toxicology Detachment Wright-Patterson AFB Dayton, OH
	Naval Medical Research Institute Detachment Lima, Peru
2000	U.S. Naval Medical Research Unit No. 2 Jakarta, Indonesia
	U.S. Naval Medical Research Unit No. 2 Detachment Manila, Republic of the Philippines
3000	U.S. Naval Medical Research Unit No .3 Cairo, Egypt
4000	Naval Biodynamics Laboratory New Orleans, LA
5000	Naval Submarine Medical Research Laboratory Groton, CT
6000	Naval Health Research Center San Diego, CA
7000	Naval Aerospace Medical Research Laboratory Pensacola, FL

Introduction

The Naval Medical Research and Development Command (NMRDC) is the primary Navy R&D organization responsible for managing research and development programs concerning the health, safety, readiness, and effective operational performance of Navy and Marine Corps personnel. The NMRDC organization consist of eight echelon-4 laboratories and five echelon-5 detachments, staffed by military and civilian scientists and located in the continental U.S. and overseas. The main research topics studies by NMRDC scientists include combat casualty care and combat dentistry, infectious diseases and AIDS, diving and submarine medicine, aviation medicine, human performance and environmental/occupational medicine.

NMRDC views the Independent Research (IR) program as unique among research programs, with its central focus on fostering the in-house laboratory investigator's scientific creativity, enthusiasm, and pride in conducting top-notch biomedical research in support of the Fleet. Through the IR program, NMRDC strives to provide these investigators with a mechanism for pursuing their novel research ideas and approaches, for broadening their expertise in state-of-the-art and emerging technologies and for initiating new efforts in research areas that are compatible with projected Navy biomedical needs. NMRDC believes the opportunities provided by the IR program are critical for stimulating and maintaining the creative and innovative thinking of Navy scientists and can only result in a heightened return for the investment of the Navy's precious basic research dollars.

"Best" Independent Research Paper of FY92

Nomination Rationale

The Naval Medical Research and Development Command (NMRDC) selected as its "best" Independent Research (IR) paper of FY92, the article entitled "Modulation of Immune Responses to Campylobacter jejuni in Mice by the Oral Administration of Cytokines." This paper describes the most recent findings of an FY90-FY92 IR project conducted by Dr. Florence Rollwagen and her associates at NMRDC's Naval Medical Research Institute (NMRI) in Bethesda, MD.

Dr. Rollwagen's project, "The Role of Lymphokines in the Generation and Maintenance of Enteric Immune Responses in Mice" investigated how cytokines effect murine immunity to C. jejuni, a bacterium recently recognized to cause acute diarrhea and bacterial gastroenteritis in military personnel throughout the world. Diarrhea is always a significant military threat during both war and peacetime operations: it is one of the major infectious diseases which cause 75 - 80% of all wartime hospital admissions and has been shown to impact almost 25% of Naval forces during overseas deployments. Cytokines (or lymphokines) are naturally secreted immune cell molecules known to be important modifiers of immune responses. Cytokine therapy currently is used for tumors, cancers, viral and other infections, but treatments usually are systemic and toxic side effects are frequent. Currently, not much is known about immunity to campylobacter, although responses at the surface of the intestinal lining (the mucosa) are thought to be important.

The concept behind this IR project was to focus research attention at this mucosal surface, defining the cytokine-modulated immune events occurring in the specific microenvironment of the campylobacter infection. This knowledge, it was believed, would be key to explaining the cytokines' role in campylobacter immunity and to exploiting these molecules for advanced prevention and treatment therapies. The study required delivering exogenous cytokines directly to the site of infection, identifying the immune cell types recruited, and assessing the nature and function of the cytokines produced by the recruited cells. Since methods for such local studies were not available, the investigators designed two novel experimental models to accomplish the investigation. One model used implanted agarose blocks infused with cytokines to study the trafficking and function of selective immune cell types (this work was previously reported in NMRDC's IR Annual Report for FY90).

The second approach involved feeding mice recombinant cytokines and assessing the cytokines' effects on the bacterial load in the intestine, lymphocyte proliferation, antibody and cytokine production, and the development of protective immunity. This approach was unique in that oral administration of cytokines had been presumed to be ineffective due to potential inactivation by stomach acids (of small intestinal tryptic peptides). Since varied biological effects were found following the oral administration of cytokines, it may now be surmised that at least some biological activity remains to effect immunological changes at the mucosal surface.

"Modulation of Immune Responses to Campylobacter jejuni in Mice by the Oral Administration of Cytokines" describes this inventive model of C. jejuni infection and immunity and implicates specific cytokines in the immune cascade leading to campylobacter immunity. Interleukin-6 (IL-6) was shown to be a potent modulator of C. jejuni infection, causing a significant increase in specific secretory IgA antibodies at the mucosal surface and an immediate (24-hour) 1000-fold

reduction in the bacterial load of the gut. Although the mechanism(s) of these cytokine effects are yet unknown, results described in this paper give promise to the potential use of cytokines as advanced therapies to prevent and control campylobacter diarrheal in Navy and Marine Corps personnel.

This work is an excellent example of the benefits brought to the Navy and its laboratories and centers through the IR program. IR support made it possible for enthusiastic Navy scientists to explore creative new approaches to a previously unanswerable questions important for the effectiveness of our Navy and Marine Corps forces. The approaches developed have not only lead to new information on local immunity to campylobacter immunity but also are being incorporated into other biomedical studies, such as wound repair research. The project has supported the graduate training of a research associate (Dr. S. Baqar, now on staff at NMRI) as well as collaborations with other biomedical scientists at the National Institutes of Health and the Uniformed Services University of the Health Sciences. It has won favorable comments on both its scientific excellence and application potential from external scientific reviewers ("the potential clinical significance is evident ... chances of obtaining relevant and practically applicable data are excellent").

The findings of this IR project have already been published in the peer-reviewed scientific literature, and have been presented at multiple scientific meetings, including three invited seminars.

Publications

Rollwagen, F. M., N. D. Pacheco, and S. Baqar. 1992. An improved model for the examination of biological effects of locally administered cytokines. J. Immun. Meth. (in press)

Rudolph, A. S. , G. Stilwell, R. O. Cliff, B. Kahn, B. J. Spargo, F. Rollwagen and R. L. Monroy. 1992. Biocompatibility of lipid microcylinders: effect on cell growth and antigen presentation in culture. Biomaterials 13:1085.

Baqar, S. 1992. Role of cytokines in Campylobacter jejuni infection and immunity in mice. Ph.D. Thesis. University of Maryland, Baltimore, MD.

Rollwagen, F. M., N. D. Pacheco, and S. Baqar. 1992. An improved model for the examination of biological effects of locally administered cytokines. NMRI Technical Report.

Presentations

Rollwagen, F. M. 1993. Cell mediated reactions: effector molecules and effector mechanisms. University of Maryland, Baltimore, MD.

Baqar, S. 1993. *The role of cytokines in infection and disease.* University of Maryland, Baltimore, MD.

Rollwagen, F. M. 1992. Administration of cytokines in agarose attracts different cell populations to the local site. Minisymposium "Cytokines in Tissues." Annual Meeting of the Federation of American Societies for *Experimental Biology, Anaheim, CA.*

Baqar, S. 1992. Mucosal and systemic immune responses to Campylobacter jejuni in mice. Minisymposium "Cytokines in Tissues." Annual Meeting of the Federation of American Societies for Experimental Biology, Anaheim, CA.

Rollwagen, F. M. 1992. Cell mediated reactions: effector molecules and effector mechanisms. University of Maryland, Baltimore, MD.

Baqar, S. 1992. The role of cytokines in infection and disease. University of Maryland, Baltimore, MD.

Spargo, B. J., A. S. Rudolph, and F. M. Rollwagen. 1993. Temporal and spatial recruitment of tissue resident cells to hydrogel composites in response to lipid microcylinders and cytokines. 19th Annual Meeting of the Society for Biomaterials, Birmingham, AL.

Spargo, B. J., G. E. Stilwell, R. O. Cliff, R. L. Monroy, F. M. Rollwagen, and A. S. Rudolph. 1991. Technological development of lipid-based microcylinders: biocompatibility and controlled release. Material Research Society Symposium on Tissue Inducing Biomaterials.

Spargo, B. J., A. S. Rudolph, and F. M. Rollwagen. 1993. Recruitment of tissue resident cells to hydrogen composites: *in vivo* response to implant materials. 4th International Symposium of Biointeractions. Molecular aspects of biomaterials, Noordwijkerhout, Netherlands.

NMRDC is very proud of the accomplishments of Florence Rollwagen, Nancy Pacheco, and Shahida Baqar and is pleased to present their work as an example of the outstanding biomedical research performed for the Navy through NMRDC's IR program.

Modulation of Immune Responses to Campylobacter Jejuni in Mice by the Oral Administration of Cytokines

S. Baqar [1], N.D. Pacheco [2] and F. M. Rollwagen [2]

[1] Geo-Centers, Inc. Ft. Washington, MD;
[2] Naval Medical Research Institute, Bethesda, MD

INTRODUCTION

Campylobacter is one of the most common bacterial infections causing acute diarrheal and bacterial gastroenteritis of military personnel throughout the world. The frequent finding of dysenteric stools suggests that mucosal damage due to an invasive process analogous to that seen in shigellosis is important in its pathogenesis (5,17). Patients infected with Campylobacter acquire specific serum IgG, IgM and IgA antibodies (6, 26). Epidemiological studies suggest that immunity to C. jejuni is acquired as a result of repeated clinical or subclinical infections (14).

Although potent antibody responses have been reported in response to C. jejuni infection, other aspects of the immune response have not been elucidated. T cell activation, estimated by in vitro lymphocyte replication assays, as well as cytokine (CK) secretion, have not been defined for this infection. CKs have been investigated as regulators of immune responses in many infectious diseases. They have been shown to be critical intermediates in the mechanisms of clinical infectious disease and the development of immunity. Exogenous CKs have been administered in vivo to enhance the immune competence of mice against infection. Interleukin (IL)-2 has been effective in protection against a wide variety of pathogens (2, 8, 16). Other CKs, such as interferon gamma (IFN-) (11), tumor necrosis factor (TNF) (22), IL-1 (19) and IL-6 (18), have also been shown to be effective.

Although administration of CKs may augment immune responses in vivo, intravenous administration has proven to be toxic, ineffective and expensive (24). Further, diffusion and half life considerations make systemic administration of CKs less than ideal. Since CKs function naturally under local conditions, regional administration avoids the problem of such materials given systemically, and can be more effective. Little or no data are available on the role of CKs in enteric infections, including Campylobacter infection, in which mucosal immunity at the local site may play an important role in protection.

We have established a model for the local administration of CKs to the mucosal surface. The aim of such treatment is the reconstitution or augmentation of naturally occurring immune responses to enteric infections such as C. jejuni.

MATERIALS AND METHODS

Study Design

BALB/c ByJ female mice, 4-6 weeks old, were fed 250-300 units of recombinant human (rh) interleukins (Genzyme, Boston, MA) in saline at 24 h and 6 h before, and once per day for 3 consecutive days after infection. Mice were infected by feeding 0.5 ml of an exponentially growing culture of C. jejuni containing 10^9 colony forming units (CFU). To determine the local and systemic immune responses, intestinal lavage fluid and serum were collected as described below.

Cultivation of Bacteria

Frozen stocks of C. jejuni strain HC were grown as previously described (7). Antigens for ELISA were prepared by sonication of organisms in log phase growth, followed by centrifugation to remove particulate cell debris. After protein determination, aliquots were frozen at -20°C until used.

Isolation of C. jejuni from Mouse Fecal Samples

Feces from individual mice were collected, rehydrated in PBS and homogenized with two wooden applicators. Serial dilutions were made in PBS and plated onto Campylobacter specific plates. After 48 h of incubation, colonies were counted and CFU per mg feces were calculated.

Sample Collection for Systemic Immunoglobulin

Systemic CK levels were determined in serum samples collected at various times post infection. Immunoglobulins were determined in the serum by ELISA.

Collection of Intestinal Lavage

At various days after challenge, intestinal secretions were collected from individual mice (12). Four doses of 0.5 ml lavage solution were given intragastrically and the mice were then given pilocarpine intraperitoneally. A discharge of intestinal contents was collected over the next 20 min. Solid material was dispersed and the samples were vortexed, then centrifuged. Supernatant fluid was collected, protein concentration determined and the samples were stored in aliquots at -70°C until assayed for immunoglobulin.

Assays for Cytokines

The concentration of CKs in serum and lavage fluid, determined in bioassays, was interpolated from standard curves using recombinant cytokines. Estimation of cell growth inhibition was made by MTT (20) uptake (IL-1) or cell proliferation by thymidine incorporation (IL-2 and IL-6). IL-1 levels were determined using the A375 cell growth inhibition assay (21). IL-2 biologic activity was determined by the IL-2 concentration dependent proliferation of a cloned murine T helper lymphocyte line HT-2 (25). IL-6 levels were determined using the B-9 cell assay (1).

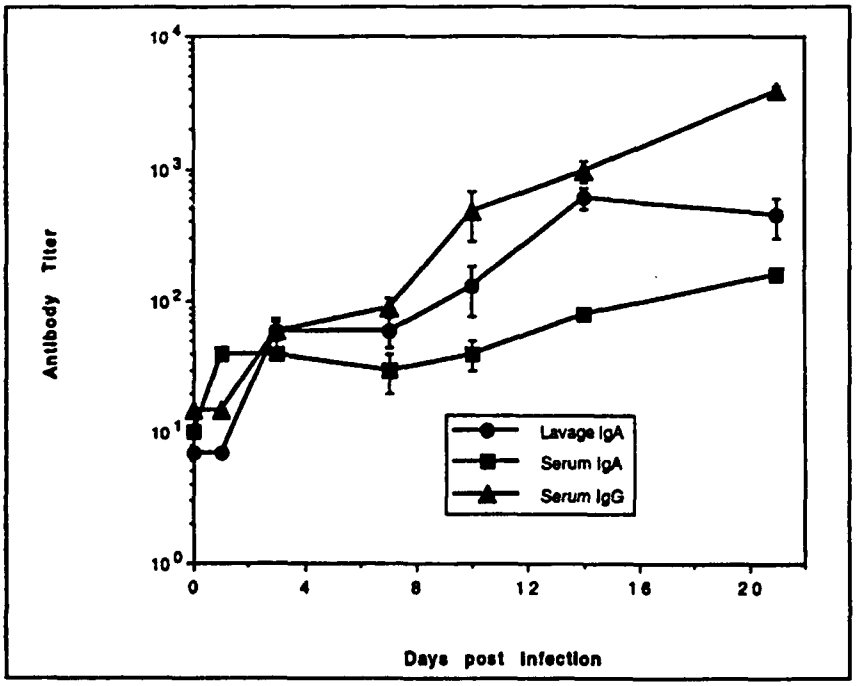


Figure 1. Mice infected with 10^9 CFU of C. jejuni were subjected to intestinal lavage or serum sampling to determine presence of antibodies. Circles = Lavage IgA, Squares = Serum IgA, Triangles = Serum IgG.

Table 1

Cytokine Levels in Lavage or Serum (Units/ml)[a]

Time Post Infection	IL-1		IL-2		IL-6	
	L	S	L	S	L	S
0 h	[b]	-	-	-	-	-
6 h	55.0	-	-	-	-	-
14 h	49.0	ND[c]	-	ND	-	ND
1 d	83.3	23.7	-	289.0	-	40.1
2 d	44.0	6.4	-	237.1	ND	0.9
3 d	59.7	-	19.1	104.0	174.0	-
6 d	-	26.5	5.1	252.0	9.8	-
10 d	64.0	ND	17.5	ND	-	ND
14 d	49.1	-	1.0	407.1	-	-
21 d	-	-	-	521.0	-	-

[a] Cytokine levels were determined in Lavage (L) or Serum (S) samples by Bioassays. Mean values of 4 mice at each time point are shown here, the standard deviation varied between 3% and 13% of the mean.

[b] Below the detection limit of the assay (0.1 u/ml)

[c] ND = not done

RESULTS

Detection of Local and Systemic Antibody Responses

Lavage IgA was detected on day 3 with a peak on day 14 as shown in Fig. 1. Serum IgA in this group was of lower magnitude, the highest level was measured on day 21. Mice showed a primary response of serum IgM which appeared at day 7, persisted for a week and decreased by day 21 (data not shown). The IgG response was first elevated on day 10 and increased to day 21 (Fig. 1). At no time in any of the groups were IgG or IgM detectable in intestinal lavage samples.

Detection of Local and Systemic Cytokines

Data presented in Table I show the concentration of CKs locally (intestinal lavage) or systemically (serum) during the course of infection. When mice were challenged with C. jejuni, local IL-1 was detected as early as 6 h post infection and remained elevated for 14 days. Systemic IL-1 in this group was transient, intermittent and only marginally elevated. Local IL-2 responses were low and transient. Systemic IL-2, however, remained elevated for an extended period of time. Local IL-6 production showed a single peak at 3 days. Serum levels of IL-6 were found to be elevated early in the infection, but they were no longer detectable by 4-6 days post last infection. No TNF or IL-4 was detected in any group at any time.

Effects of Cytokines on the Course of C. jejuni Infection in Mice

In a series of preliminary experiments, mice fed supernatant fluids from Con A stimulated rat spleen cells (CAS) showed a reduction of C. Jejuni in feces and an augmented immune

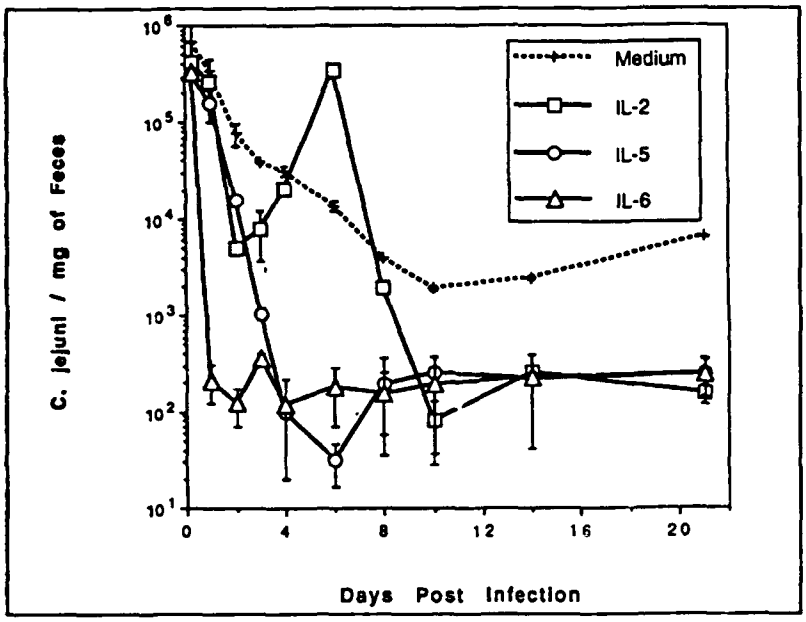


Figure 2. Mice were fed recombinant IL-2 (open squares), IL-5 (open circles), IL-6 (open triangles), or culture medium (dashed line) as a control. They were then infected with C. jejuni. At intervals post infection the number of colonies of C. jejuni/mg feces was determined.

response to C. jejuni specific antigens. Recombinant murine IL-2, IL-5 and IL-6 were selected for use based on their presence in the CAS as well as their role in generation of the cellular and humoral immune responses during an enteric infection. At various intervals starting 6 h post infection, fecal samples were collected for quantitative determination of C. jejuni/mg feces. Data presented in Fig. 2 show that the initial colonization of bacteria in the gut of untreated or cytokine treated mice was similar, averaging 5 x 10^5 CFU/mg of feces. Mice treated with IL-6 showed an immediate and the most pronounced effect of the treatment. Within 24 h, the bacterial load in the gut was reduced by at least 3 logs when compared with untreated controls, and this difference was maintained with very little or no variation for 21 days even though the treatment was stopped after 3 days. Effects of IL-5 treatment on the gut colonization by C. jejuni was gradual although by day 6 the average number of bacteria per mg of feces in this group had

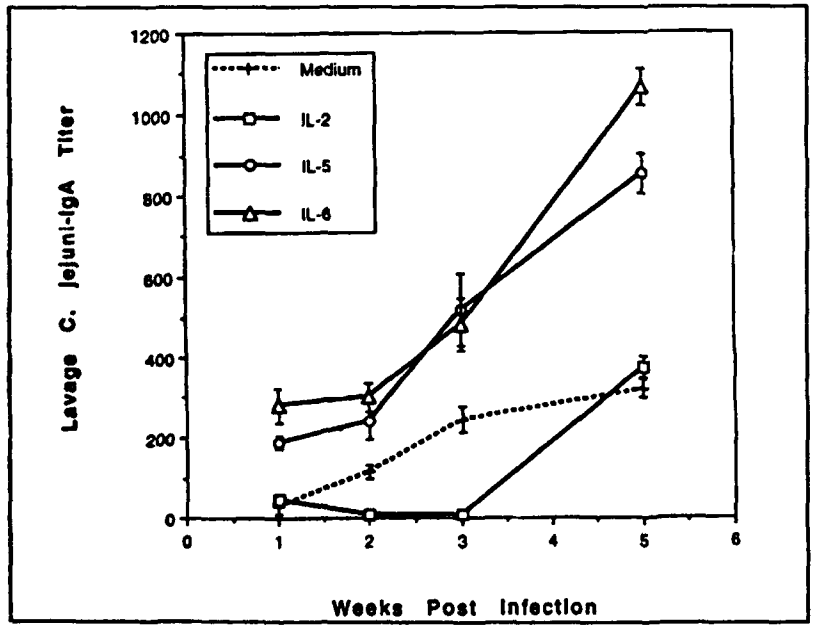


Figure 3. Mice were fed recombinant CKs then infected with C. jejuni. At intervals, intestinal lavage fluid was collected for estimation of antigen specific IgA titers. Symbols are the same as in Figure 2.

dropped to the IL-6 level. Effects of IL-2 treatment were delayed, and for 6-8 days the average number of bacteria in feces of the IL-2 treated group was either similar to or higher than the control group. At day 8, the IL-2 treated group exhibited a reduction (1.5 log) in the bacterial load and, by day 10, it had dropped to the level seen with other cytokines.

Effects of Cytokines on the Generation of Humoral Immune Responses to C. jejuni

The kinetics of generation of C. jejuni specific circulatory or intestinal lavage IgA or IgG were determined by ELISA using sonicated bacteria as antigen. End point IgA titers in lavage from mice treated with IL-2, IL-5, IL-6 and medium controls are presented in Fig. 3. It is evident that IL-5 or IL-6 treatment of mice resulted in an enhanced secretory IgA production which was initiated at least 1 week post infection. IL-2 appeared to have little effect on IgA production, since titers were the same or lower than medium controls.

IL-2 feeding resulted in enhanced serum IgA antibodies to C. jejuni at 14 days after infection (Fig. 4), however this effect on IgA was transient and titers by day 21 fell to control levels (1:340). IL-6 treatment of mice gave the most pronounced and sustained effect on levels of IgA in the circulation.

DISCUSSION

A murine model was developed to study immune and inflammatory responses to C. jejuni infection. This model also was used to evaluate the effects of CK administration to the gut mucosal surface. The host humoral immune response and the CK secretion profiles following C. jejuni infection were characterized. It was found that antigen-specific humoral immune responses developed in response to C. jejuni infection in mice. CKs were detected in lavage following infection. IL-1 and IL-6 were found, but not IL-2. Serum CK levels revealed the presence of early intermittent low levels of IL-1 and IL-6. IL-2 levels were markedly elevated and sustained. Following this pattern of CK secretion in natural infection, we administered recombinant CKs in order to modulate the generation of protective immunity. Such treatment resulted in enhanced humoral immunity to C. jejuni.

In the present study, administration of IL-6 resulted in an augmentation of host defenses which led to early elimination of bacteria. In addi-

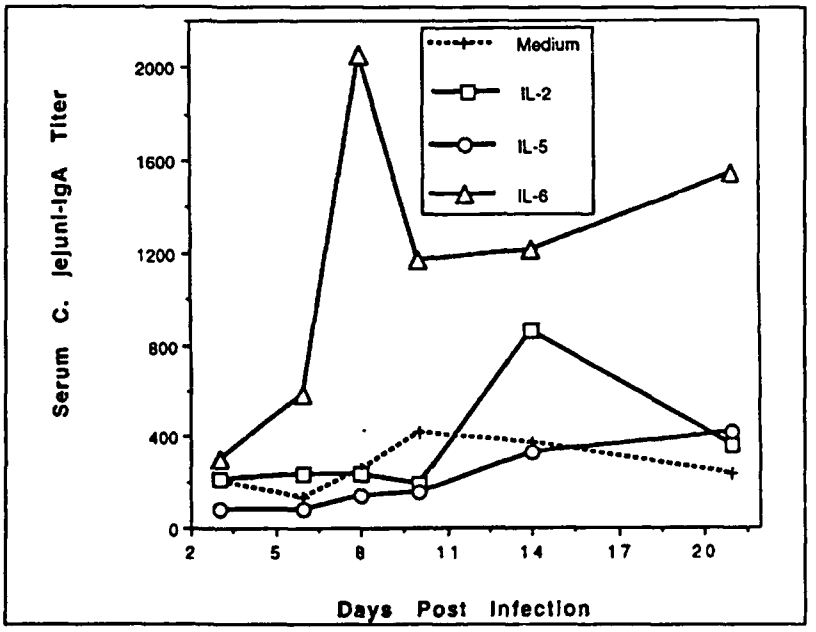


Figure 4. Mice were treated as in Figure 2 and Figure 3. Serum was obtained at intervals post infection. Symbols are the same as in Figure 2.

tion to IL-6 effects, IL-5 or IL-2 administration induced a delayed effect on the course of infection. It appears that CK administration had an adjuvant-like effect on the development of the immunity to C. jejuni in mice.

CK administration in vivo has varied effects on the course of infection in a number of models. Degre et al. (10) reported that pretreatment of mice with TNF-alpha 24 h before the administration of S. typhimurium reduced the establishment of intracellular infection in the intestinal epithelial cells and the development of bacteremia. Intravenous administration of IL-1 before, but not after, infection suppressed the growth of B. abortus in the spleens and livers of mice (27). Similar effects were observed when IFN- was used for other Brucella infections, suggesting that quite early events determine the outcome of the infection. Others have shown that IL-1 given before injection of mice with any one of a number of pathogenic bacteria enhances resistance to subsequent infection (9, 23). A recent report (18) has shown the IL-6 injected intraperitoneally before infection with L. monocytogenes protects mice in a dose dependent manner. Contrary to these studies, it has been reported that Con A or LPS induced spleen cell supernatant fluid had no influence on T. brucei growth under axenic culture conditions over a wide range of concentrations (3). Recent results have suggested that orally administered IFN- can affect peripheral white blood cell numbers (13). All types of white blood cells were shown to be reduced. These data suggest that orally administered CKs may have profound systemic effects. Results in our laboratories and in others (13), which have shown that CKs may not be detectable in the circulation of these mice, suggest that CKs effects may be mediated by cell subpopulations or by secondary CK secretion in target organs.

None of the CK's is known to have direct bacteriostatic or bactericidal effects in vivo. The actual mechanism(s) of this clearance is not known but early and transient CK production following infection, as well as its therapeutic effect, suggest that IL-6 plays an important role in early priming of resistance. An augmented C. jejuni specific IgA response in IL-6 treated mice suggests that IL-6 is responsible for the rapid secretion of IgA. The detection of IL-6 in intestinal lavage indicates that it is a potent intermediary in this response.

Clinical symptoms of C. jejuni infection include fever and diarrhea. Our finding that IL-1 is secreted locally early post infection is consistent with the clinical symptoms of fluid loss resulting in diarrhea. Invasion of C. jejuni into the circulation results in systemic IL-1 secretion and may be responsible for the generation of fever. The appearance in the circulation of both IL-1 and IL-6 may be a response to this translocation from the intestine. Other models of infection have shown that circulating lymphocytes secrete IL-6 in vitro when stimulated with M. avium antigens (4). The role of IL-6 in immunity and pathogenesis is not clear. It has been described as a growth promoter for B-cells, as well as an inducer of inflammation. Our data support the finding that IL-6 is generated as part of an ongoing mucosal immune response to bacteria. The spectrum of biological activities of IL-6 makes it a likely mediator, from the mucosal site of infection to the systemic compartments, responsible for some signs and symptoms of the infection. The urinary tract infection model has provided evidence for the segregation of mucosal and systemic IL-6 production (15). Elevation of circulating IL-6 did not lead to the excretion of IL-6 into the urine, and urinary IL-6 secretion in response to mucosal challenge preceded the systemic response.

With the increasing availability of recombinant CKs, the possibility of their therapeutic use is causing increased interest in their protective role in infection. The data presented in this study suggest that IL-1, IL-2, IL-5 or IL-6 have different mechanism(s) for induction and regulation of the protective immune response to an infectious

agent. Currently we are investigating the mechanism(s) involved in developing such immunity in C. jejuni infections of mice.

REFERENCES

1. Aarden, L.A.; E.R. De Groot, O.L. Schaap, and P.M. Lansdorp. 1987. Production of hybridoma growth factor by human monocytes. Eur. J. Immunol. 17:1411-1416.

2. Bermudez, L.E.M, P. Stevens, P. Kolonoski, M. Wu, and L.S. Young. 1989. Treatment of experimental disseminated Mycobacterium avium complex infection in mice with recombinant IL-2 and tumor necrosis factor. J. Immunol. 143:2996-3000.

3. Black, S.J., M. Murray, S.Z. Shapiro, R. Kaminsky, N.K. Borowy, R. Musanga, and F. Otieno-Omondi. 1989. Analysis of Propionibacterium acnes-induced non-specific immunity to Trypanosoma brucei in mice. Parasite Immunol. 11:371-383.

4. Blanchard, D.K., M.B. Michelini-Norris, C.A. Pearson, C.S. Freitag, and J.Y. Djeu. 1991. Mycobacterium avium-intracellular induces interleukin 6 from human monocytes and large granular lymphocytes. Blood. 77:2218-2224.

5. Blaser, M.J., I.D. Berkowitz, F.M. LaForce, J. Cravens, L.B. Reller, and W.L. Wang. 1979. Campylobacter enteritis: clinical and epidemiological features. Ann. Intern. Med. 91:179-185.

6. Blaser, M.J., D.J. Duncan, M.T. Osterholm, G.R. Istre, and W.L. Wang. 1983. Serologic study of two clusters of infection due to Campylobacter jejuni. J. Infect. Dis. 147:820-823.

7. Caldwell, M.B., R.I. Walker, S.D. Stewart, and J.E. Rogers. 1983. Simple adult rabbit model for Campylobacter jejuni enteritis. Infect. Immun. 42:1176-1182.

8. Chong, K-T. 1987. Prophylactic administration of interleukin-2 protects mice from lethal challenge with gram-negative bacteria. Infect. Immun. 55:668-673.

9. Czuprynski, C.J., J.F. Brown, K.M. Young, A.J. Cooley and R.S. Kurtz. 1988. Effects of murine recombinant interleukin 1 on the host response to bacterial infection. J. Immunol. 140:962-968.

10. Degre, M. and G. Bukholm. 1990. Effect of tumor necrosis factor-alpha on infection with Salmonella typhimurium in a mouse model. J. Biol. Regul. Homeost. Agents. 4:157-161.

11. Edwards,III, C.K., H.B. Hedegaard, A. Zlotnik, P.R. Gangadharam, R.B. Johnston, Jr., and M. J. Pabst. 1986. Chronic infection due to Mycobacterium intracellular in mice: Association with macrophage release of prostaglandin E_2 and reversal by injection of indomethacin, muramyl dipeptide, or interferon-. J. Immunol. 136:1820-1827.

12. Elson, C.O., W. Ealding, and J. Lefkowitz. 1984. A lavage technique allowing repeated measurement of IgA antibody in mouse intestinal secretions. J. Immunol. Meth. 67:101-108.

13. Fleischmann, Jr., W.R., S. Koren and C.M. Fleischmann. 1992. Orally administered interferons exert their white blood cell suppressive effects via a novel mechanism. Proc. Soc. Exp. Biol. and Med. 201:200-207.

14. Glass, R.I., B.J. Stoll, M.I. Huq, M.J. Struelens, M. Blaser, and A.K.M.G. Kibriya. 1983. Epidemiologic and clinical features of endemic Campylobacter jejuni infection in Bangladesh. J. Infect. Dis. 148:292-296.

15. Hedges, S., P. Anderson, G. Lidin-Janson, P. de Man, and C. Svanborg. 1991. Interleukin- 6 response to deliberate colonization of the human urinary tract with gram-negative bacteria. Infect. Immun. 59:421-427.

16. Iizawa, Y., T. Nishi, M. Kondo, K. Tsuchiya and A. Imada. 1988. Effect of recombinant human interleukin-2 on the course of experimental chronic respiratory tract infection caused by Klebsiella pneumonia in mice. Infect. Immun. 56:45-50.

17. Klipstein, F.A., R.F. Engert, H. Short, and E.A. Schenk. 1985. Pathogenic properties of Campylobacter jejuni: Assay and correlation with clinical manifestations. Infect. Immun. 50:43-49.

18. Liu, Z., R.J. Simpson and C. Cheers. 1992. Recombinant interleukin-6 protects mice against experimental bacterial infection. Infect. Immun. 60:4402-4406.

19. Morrissey, P.J. and K. Charrier. 1991. Interleukin-1 administration to C3H/HeJ mice after but not prior to infection increases resistance to Salmonella typhimurium. Infect. Immun. 59:4729-4731.

20. Mosmann, T. 1983. Rapid colorimetric assay for cellular growth and survival: Application to proliferation and cytotoxicity assays. *J. Immunol. Meth* 65:55-63.

21. Nakano, K., K. Okugawa, H. Hayashi, S. Abe, and Y. Sohmura. 1987. Establishment of dye-uptake method (A375 assay) for quantitative measurement of IL-1: Correlation with LAF assay. Dev. Biol. Stand. 69:93-101.

22. Nakano, K, K. Onozuka, Y. Terada, H. Shinomiya and M. Nakano. 1990. Protective effect of recombinant tumor necrosis factor- in murine Salmonellosis. J. Immunol. 144:1935-1941.

23. Ozaki, Y., T. Ohashi, A. Minami, and S-I. Nakamura. 1987. Enhanced resistance of mice to bacterial infection induced by recombinant human interleukin 1. Infect. Immun 55:1436-1440.

24. *Roth, A.D., and J.M. Kirkwood. 1989. New clinical trials with interleukin-2: Rationale for regional* administration. Nat. Immun. Cell Growth Regul. 8:153-164.

25. Watson, J. 1979. Continuous proliferation of murine antigen-specific helper T lymphocytes in culture. J. Exp. Med. 150:1510-1519.

26. Watson, K.C., E.J.C. Kerr and S.M. McFadzean. 1979. Serology of human Campylobacter infection. J. Infect. 1:151-158.

27. Zhan, Y.F., E.R. Stanley and C. Cheers. 1991. Prophylaxis or treatment of experimental brucellosis with interleukin-1. Infect. Immun. 59:1790-1794.

The Genetics of Motion Sickness

Warren Lockette, M.D.[1], and Neil Shepard, Ph.D.[2]

[1]Naval Health Research Center, San Diego, CA
[2]Wayne State University School of Medicine, Detroit, MI
University of Michigan Medial School, Ann Arbor, MI

ABSTRACT

Motion sickness and vestibular disorientation are unpleasant experiences that, depending upon the severity of the provocation, affect a large percentage of the general population and decrease task performance by military air, diving, and shipboard personnel. The mechanism(s) by which adverse physiologic responses occur during provocative motion are believed to be similar. A search for the etiology of motion sickness has been underway for a number of years; we propose a new approach. We postulate that a propensity for motion sickness is a heritable trait. By first identifying the gene(s) associated with the development of motion sickness, and then by recognizing the product of these genes, the physiology underlying the development of motion sickness can be understood.

Specifically, we hypothesized that genetic polymorphism of the alpha-2 adrenergic receptor gene may be associated with a predisposition for motion sickness in healthy subjects. We challenged our hypothesis by studying a discrete group of subjects whose relative sensitivity and resistance to provocative motion is well characterized. Coriolis stress susceptibility (CSSI) was measured on 45 volunteers with normal vestibular function. CSSI was measured by determining the number of head movements subjects could complete while being rotated at increasing velocity before they developed motion sickness. Genomic DNA was isolated from the leukocytes of these individuals and digested with a restriction enzyme, Dra I. Southern analysis using a 4.5 Bam HI probe complementary to the alpha-2 adrenergic receptor gene on chromosome 10 yielded a restriction fragment length polymorphism (RFLP) of 6.3 and 6.7 kB. Individuals heterozygous for the 6.7/6.3 alleles were significantly more prone to the development of motion sickness in response to Coriolis stress compared to 6.7 homozygotic individuals ($p < 0.1$); no 6.3/6.3 homozygotes were found. We have cloned the region responsible for this polymorphism; it is found in the 3' portion of the gene that may control mRNA stability. Polymorphism of this gene results from a uniform A to G substitution approximately 800 bases downstream from the last translated codon of this single exon gene. Genetic differences in the alpha-2 adrenergic receptor that mediate the release of catecholamines in the central nervous system may predispose an individual to motion sickness. Study of other candidate genes and reverse genetics may help explain the variation of responses to untoward motion elicited from an individual as well as the differences among the population.

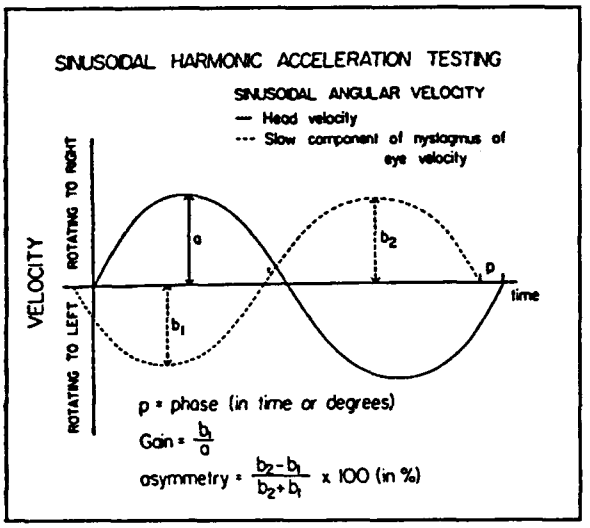


Figure 1. The semi-circular canals sense angular acceleration. When the head is rotated, the eyes move to maintain the visual field. Nystagmus is a reflex response that requires intact sensori-neuronal pathways between the inner ear, the brainstem, and oculomotor nuclei. Because the cornea has an electric potential relative to the retina, electrodes were placed on the subject's forehead; the compensatory eye movements were recorded in the dark as changes in the potential differences. Abnormalities in any of these pathways may result in alteration in the gain, phase, or symmetry of the compensatory eye movements in response to rotation. The phase is the time delay between head movement and eye movement; the gain is the average peak velocity of eye movements to the velocity of head movements; symmetry measures the peak velocity of the compensatory eye movements following rotation to the right compared to the velocity of the compensatory eye movements following rotation to the left.

BACKGROUND

The high prevalence of motion sickness is of operational concern to all branches of the Department of Defense, and as recently as 1992, a number of studies have documented an unacceptably high incidence of motion sickness in aircrew and shipboard personnel (1-6). Furthermore, it has recently been recognized that several aspects of the underwater environment such as sensory conflicts, body fluid redistribution, and nitrogen narcosis also make the Naval diver more susceptible to motion sickness (7). The mechanism(s) by which adverse responses occur to provocative motion is unknown.

A number of theories have been proposed to account for the development of motion sickness, and they have been extensively reviewed. It was once believed that motion sickness resulted from untoward motion to the vestibular system. However, a number of unexplained observations, described by Oman (8), resulted in the rejection of this theory: Learning and adaptation to provocative motion can occur; people frequently stimulate their vestibular system without untoward responses; motion sickness can occur in the absence of movement (e.g. optokinetic stimuli); and active cognition decreases the symptomatology of motion sickness. Most recently, the understanding of motion sickness has evolved into the more tenable sensory conflict model. In this paradigm, sea sickness, car sickness, air sickness, spectacle sickness, flight simulator sickness, space adaptation syndrome, and vestibular disorientation are considered to have the same origin. Oman, and others, have proposed that motion sickness symptoms result whenever there is discrepant information given to us by one set of sensations and that given to us by another set of stimuli. It is proposed that specific neurons and paracrine factors originating in the regions of the brain responsible for spatial orientation send messages to those regions mediating motion sickness symptoms (8). This hypothesis has been extensively modeled, it is in

agreement with quantitative studies on motion sickness, and it is in harmony with our theory of a genetic basis for motion sickness.

We are testing our general hypothesis that there is a genetic propensity for the development of motion sickness, and by using a "candidate" gene approach, we can identify the mechanisms(s) by which motion sickness develops. We present preliminary data in support of our hypothesis. The work we have begun is in a nascent field of development. We can find no study proving that the propensity for motion sickness is a heritable trait. It is suggested from reports in the basic science literature that the possibility of a genetic susceptibility to motion sickness has been overlooked (9,10). For example, the squirrel monkey is often used to study motion sickness, and scientists report marked strain differences in the susceptibility to motion sickness in these non-human primates. Specifically, the Bolivian strain (Saimiri sciureus boliviensis) is exquisitely sensitive to provocative motion, whereas the Brazilian strain (Saimiri sciureus sciureus) is relatively refractory to the development of motion sickness. These New World monkeys are clearly inbred strains; they are under similar environmental influences; and they demonstrate other genetic characteristics that segregate as Mendelian traits in closed populations. In man, ethnic differences in susceptibility to motion sickness have been reported, but there has been no speculation on the reasons for these racial discrepancies (11).

Our hypothesis that motion sickness is a heritable trait in man evolved out of our fortuitous observation that individuals with pathologic elevations in blood pressure complain more frequently of motion sickness than normotensive volunteers. We demonstrated that individuals with essential hypertension were more likely to develop motion sickness, and in the conduct of this study, we noted a bimodal distribution of tendency towards motion sickness in our normotensive subjects.

To determine the predisposition of the individuals for motion sickness, we performed Coriolis stress testing on asymptomatic subjects with essential hypertension and a group of normotensive volunteers. The Coriolis effect is evoked when an individual moves out of a plane of rotation, and it is an extremely provocative, frequently used, and reproducible stimuli for inducing motion sickness.

Before measuring Coriolis stress susceptibility, we first ensured that none of our subjects had underlying vestibular dysfunction by measuring their response to sinusoidal harmonic acceleration (Figure 1) using a computerized rotational chair. Subjects were seated in a light-tight enclosure on a rotational chair mounted on a DC-torque motor controlled by a DEC computer. The chair underwent sinusoidal harmonic acceleration at three frequencies with a peak angular velocity of 50°/sec at each frequency and peak angular accelerations of 3, 12, and 50°/sec. There were no differences in the gain, phase, and symmetry of the vestibulo-ocular reflex between the normotensive and hypertensive volunteers. All subjects had "normal" vestibular function by these standard studies (12).

Coriolis stress susceptibility was then measured by determining the number of head movements that a subject could make in the dark with no visual clues while they were rotated in a chair at increasing velocity. Every eight seconds, the subject was asked to lean 45° forward, backward, left, and right from the vertical axis of the chair. The subjects were rotated initially at 1 rpm, and the chair speed was increased by 2 rpm every time the subject completed eight sets of head movements. The subjects were queried about their symptoms of motion sickness after each set of head movements, and they received 1 to 3 points for each symptom developed: dizziness, headache, drowsiness, cold sweats, subjective warmth, increase in salivation, epigastric awareness or distress, and nausea. The test was terminated when the subject obtained eight points or when the chair speed reached 23 rpm.

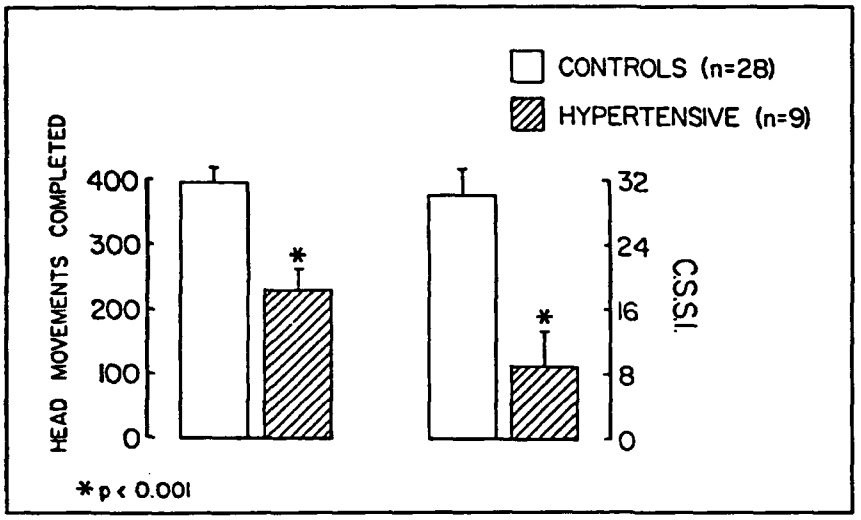
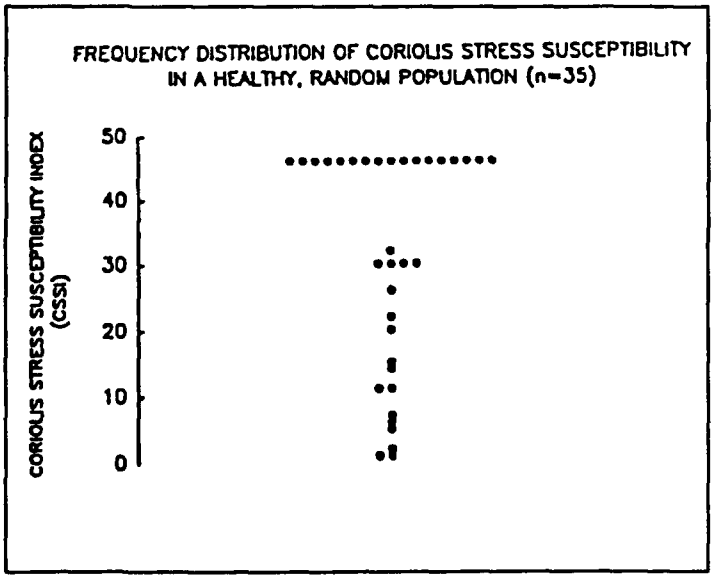


Figure 2. Normal subjects were able to complete more (386± 28) head movements before reaching the pre-determined endpoint malaise compared to the hypertensive subjects (219± 21), and accordingly had higher Coriolis Stress Susceptibility Index scores (CSSI, p<0.001, Ref. 12).

Many subjects were studied on two occasions. Although some persons develop adaptive behavior that decreases the symptoms of motion sickness ("getting one's sea legs"), the Coriolis stress test has the advantage that very little learning occurs during this limited exposure. As a result, the number of head movements that an individual can perform before the development of motion sickness is highly reproducible.

All of our hypertensive subjects, who were otherwise healthy, developed motion sickness quite easily (Figure 2). However, nearly a quarter to one half of the normotensive subjects were able to complete all of the head movements at the maximum chair velocity; none of the hypertensive individuals were able to complete the Coriolis stress test before reaching the predetermined endpoint malaise. Furthermore, the susceptibility to motion sickness in hypertension was much greater than suggested by these data. As the chair speed increases, the Coriolis stress experienced with each head movement becomes even greater. Accordingly, those individuals able to perform more head movements at faster rotational velocity experienced greater Coriolis stress than is reflected by the actual number of head movements. A Coriolis stress susceptibility index has been developed that mathematically accounts for the increased chair speed with succes-

Figure 3. Coriolis stress susceptibility was measured in 35 healthy, normotensive subjects. The CSSI was calculated from the number of head movements an individual seated in a rotating chair could make out of the plane of motion. Between 25-50% of the subjects tested never developed symptomatology of motion sickness. Although almost anyone can be made sick given a severe enough provocation, there is clearly a marked difference between the two ends of the spectrum, i.e. individuals with very high, or very low, tolerance.



sive increases in the number of head movements (13,14). The subjects with essential hypertension had extremely low CSSI scores which reflected their marked susceptibility to motion sickness. Why were some of the normotensive individuals apparently immune to untoward response of this provocative stimuli?

During our studies, we found a bimodal distribution of susceptibility to Coriolis stress in healthy individuals with normal blood pressures. Approximately 25-50% of our normal subjects were immune to the development of motion sickness induced by Coriolis stress. Using different provocative stimuli such as vection, others have reported a similar bimodal trend in succumbing to motion sickness. Although individuals may have contrasting susceptibility to motion sickness, there are clearly two populations of individuals—those who develop motion sickness and those who generally do not. It is suggested from these observations that the predisposition for motion sickness can be inherited as a simple trait in Mendelian manner (Figure 3).

Finally, in these preliminary studies, we studied three sets of normotensive, monozygotic twins whose lineage was verified by DNA fingerprint analysis. Both sibs in all three sets of twins had nearly identical CSSI scores! It is suggested from this high concordance rate that susceptibility to motion sickness has a strong genetic component. Accordingly, we hypothesize that susceptibility to Coriolis stress is a heritable trait, and we have begun to identify those genes responsible for untoward responses to provocative motion.

METHODS

There are three methods by which genetic analysis can be used to identify the gene(s) associated with a propensity for motion sickness. Classic genetic linkage analysis identifies the effects of a single gene on a well-defined, measurable, physical characteristic, i.e. the phenotype (15). In simple segregation analysis, one tests the hypothesis that the pattern of disease inheritance fits that predicted by a Mendelian, single gene, model (15). Family linkage, or pedigree, analysis, is then used to find the location of a gene (the locus), related to a particular trait, in the genome (the chromosomes). In a linkage study, family members undergo "typing" for a series of polymorphic markers and disease phenotype. These polymorphic markers are different alleles (different forms of the same genes within the population). If a disease phenotype (in this case, susceptibility to motion sickness) and a genetic marker (our "candidate

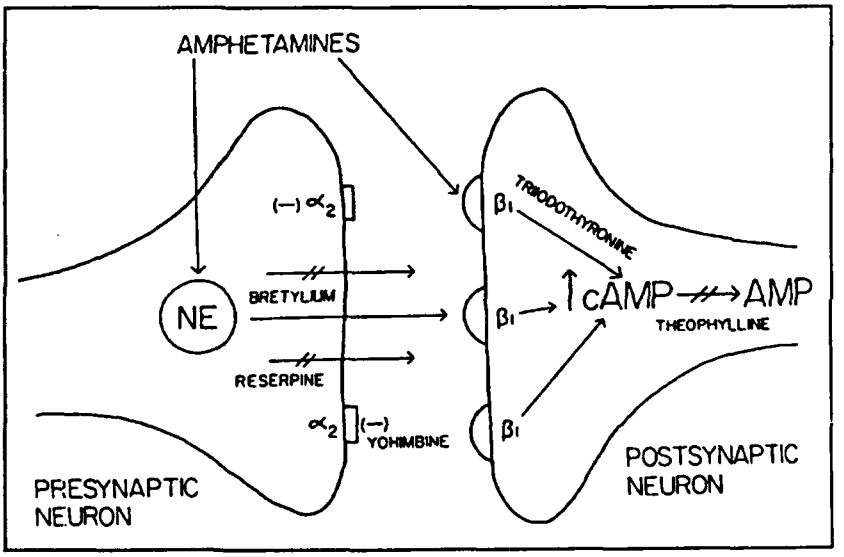


Figure 4. The alpha-2 adrenergic receptor mediates catecholamine release. Norepinephrine (NE), released from pre-synaptic neurons, binds to post-synaptic alpha-1 and alpha-2 receptors. The NE released also binds to pre-synaptic alpha-2 adrenergic receptors in a negative feedback loop to prevent the further release of NE (16,17).

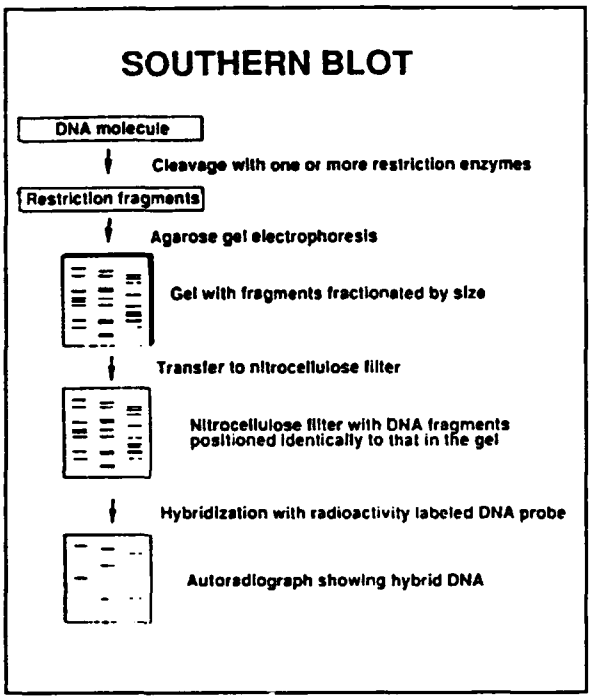


Figure 5. Genomic DNA, isolated from leukocytes or tissue of an individual, is digested with restriction endonucleases that cleave DNA between specific sequences. The restriction fragments are fractionated by size using gel electrophoresis. The DNA is then denatured into single strands and transferred to a nylon filter. Radioactively labeled probes of single stranded DNA are next added to the filter, and hybridization between complementary sequences of DNA are visualized by placing the filter over x-ray film. This technique where DNA is run on an electrophoresis gel and then transferred to a nylon filter is known as Southern blotting.

gene") segregate (pass) through the family together more often than expected by chance alone, the disease locus and the genetic marker are said to be linked. If family members who inherit the disease (i.e. susceptibility to motion sickness) almost always inherit the same genetic marker, then these two loci are said to be tightly linked (15). This type of study is done without difficulty when the phenotype is a specific trait, and it is not subject to acute, nongenetic, external influences such as learning, physical training, or the environment. Alternatively, association studies are capable of detecting genes that exert some effect on complex quantitative traits (14). Prospective genes that may exert an influence on susceptibility to motion sickness can be known ("candidate genes") or yet unidentified ("anonymous"). We hypothesized that a genetic polymorphism of the alpha-2 adrenergic receptor, a candidate gene, is associated with a predisposition for the development of motion sickness. The alpha-2 adrenergic receptors are found on pre-synaptic neurons of the central and peripheral nervous system, and they modulate the release of catecholamines from noradrenergic nerve terminals (Figure 4).

Our hypothesis that a genetic polymorphism of this candidate gene is associated with the susceptibility to Coriolis stress is based upon the following observations: (1) Agents which provoke the release of catecholamines centrally are the most potent agents at decreasing the signs and symptoms of motion sickness. (2) Physical training in endurance sports increases susceptibility to motion sickness, and we have demonstrated that endurance training results in an up-regulation and increase in pre-synaptic alpha-2 adrenergic receptors and a decrease in catecholamine release in man. (3) Aroused, attentive states increase central catecholamines, and this cognition decreases susceptibility to motion sickness. (4) Individuals with essential hypertension have a markedly increased sensitivity to Coriolis stress, and we have demonstrated that a polymorphism of the alpha-2 adrenergic receptor is strongly associated with essential hypertension in man. (5) Motion sickness is associated with increases in plasma vasopressin, corticotropin hormone releasing factor, and cortisol in man; the alpha-2 adrenergic receptor mediates the central release of these hormones and releasing factor. (6) Changes in the number of adrenergic and cholinergic receptors on CNS neurons mediate learning; adaptation to untoward motion stimuli can occur; and this learning may be temporally related to changes in the receptor complement

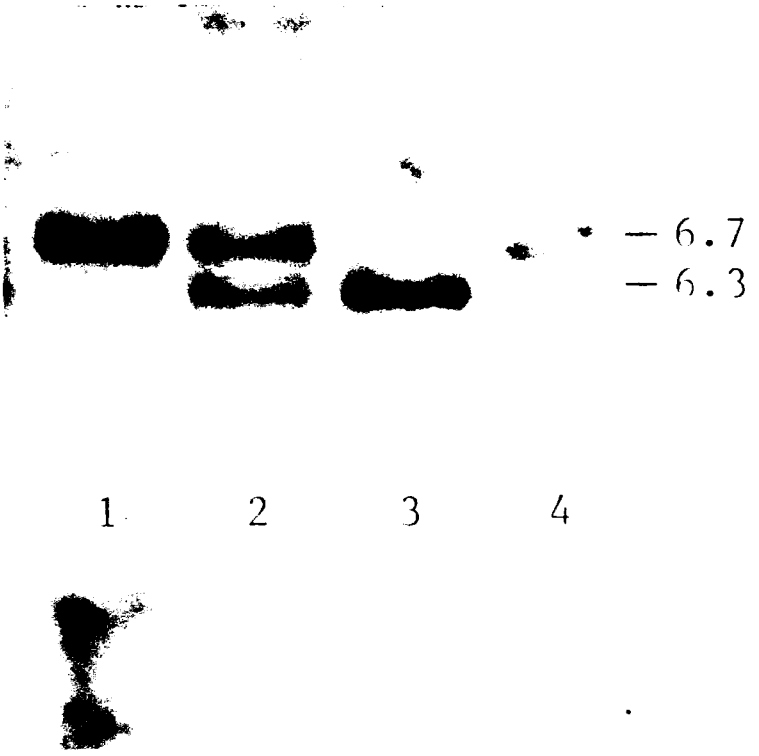


Figure 6. Polymorphism of the C10 alpha-2 adrenergic receptor. Genomic DNA was randomly obtained from four subjects, digested with the restriction enzyme Dra I, and probed with a 5.5 kB probe for the chromosome 10 alpha-2 adrenoreceptor. Dra I identified a RFLP of 6.3 and 6.7 kB of the alpha-2 adrenergic receptor gene on chromosome 10 in humans. Lane 1, homozygote for the 6.7 allele; Lanes 2,4, co-dominant heterozygote; Lane 3, 6.3 homozygote. It is also significant that the Dra-1 site is in a noncoding, regulatory region of the gene.

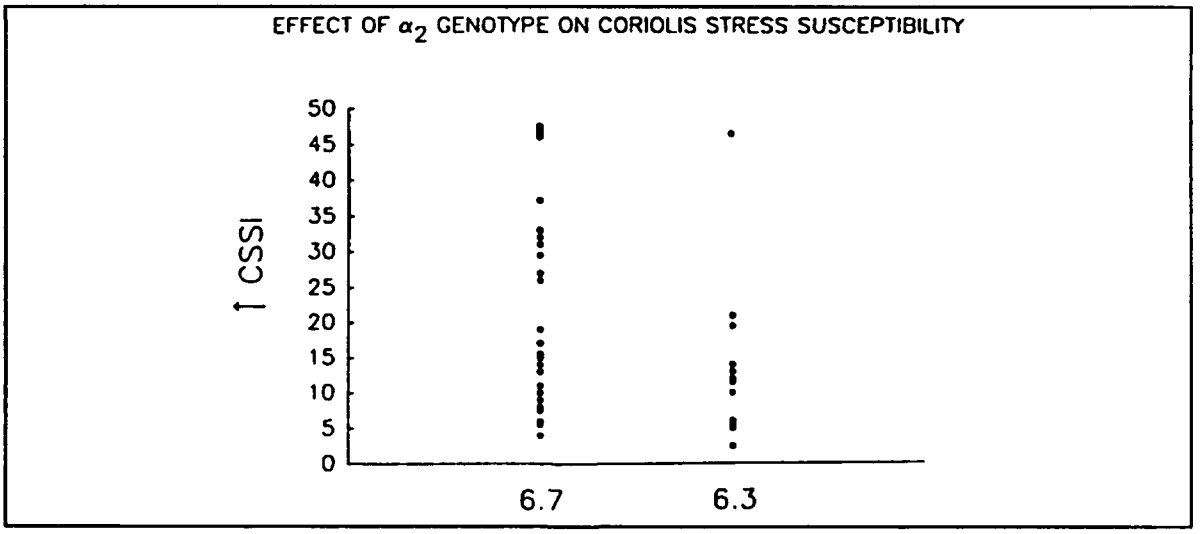


Figure 7. 39 men and women were studied. Individuals refractory to the development of motion sickness had higher Coriolis stress susceptibility index (CSSI) scores. Individuals homozygous for the 6.7 allele ("6.7") experienced varying degrees of motion sickness. Heterozygotes for this allele, i.e. having at least one 6.3 allele ("6.3"), as a group, were more likely to develop motion sickness at a significantly lower level of Coriolis stress. We reported that homozygosity for the 6.3 allele is associated with the development of essential hypertension. Since this current study included only healthy subjects without hypertension, it was not surprising that no 6.3/6.3 homozygotes were found in our random selection of subjects from the healthy population.

of these neurons. (7) Central alpha-2 adrenergic receptors modulate peripheral vagal tone; and motion sickness is associated with an increase in parasympathetic, vagal activity (16-25).

The position of a gene on a chromosome is known as the locus. An individual has two homologous chromosomes, i.e. one chromosome is inherited from each parent. Accordingly, since each chromosome carries a particular gene, an individual receives one copy of the gene from the mother and one from the father. Variations may exist between the same gene locus on homologous chromosomes, and these different forms of the same gene are known as alleles. Genetic polymorphism refers to the significant presence of two or more different alleles within a population. Since an individual inherits a maternal and paternal chromosome, he/she will inherit two alleles which may, or may not, be identical. Genetic polymorphisms of these alleles can be identified using the techniques of Southern blotting and restriction fragment length polymorphism (Figure 5).

RESULTS

A gene encoding the alpha-2 adrenoreceptor on chromosome 10 is polymorphic in the population. Cleavage of genomic DNA with the restriction enzyme Dra I and subsequent hybridization with a cDNA probe reveals two alleles of varying size, one 6.3 kB and the other 6.7 kB (Figure 6). Coriolis stress susceptibility was measured in 39 volunteers with normal vestibular function by determining the number of head movements subjects could complete while being rotated at increasing velocity before they developed motion sickness. Genomic DNA was isolated from their leukocytes, digested with Dra I, and hybridized to a cDNA probe as we have described (20,26,27).

Southern analysis using a 4.5 Bam HI probe complementary to the C10 alpha-2 adrenergic receptor gene yielded a restriction fragment length polymorphism of 6.3 and 6.7 kB. Individuals heterozygous for the 6.7/6.3 alleles had significantly lower CSSI scores (p) compared to the 6.7 homozygotic individuals; no 6.2/6/3 homozygotes were found (Figure 7). Alpha-2 adrenergic receptors modulate central catecholamine concentrations, and genetic differences in central catecholamine release may predispose an individual to motion sickness.

The mechanism(s) by which a genetic polymorphism of the C10 alpha-2 adrenoreceptor can be associated with hypertension or susceptibility to motion sickness is unknown. We have mapped this polymorphism to the 3' noncoding portion of the C10 alpha-2 adrenergic receptor gene. Unless this polymorphism is accompanied by a mutation in the coding portion of this gene, it is unlikely that this mutation results in a change in the binding constants of the alpha-2 adrenergic receptor. However, it has been demonstrated that mutations in the 3' portion of many genetic loci contribute to the stability of the complementary mRNA encoded by the gene, and such a mutation could affect protein translation, and therefore receptor number. It is also interesting that the polymorphism of the C10 alpha-2 adrenergic receptor gene involves loss of a Dra-I recognition site (TTTAAA), and the sequence ATTTA encodes a rapid degradation signal in complementary messenger RNA (28). Aberrations of an individual's ability to modulate his/her complement of alpha-2 adrenergic receptors could explain individual differences in central catecholamine concentrations and adaptation to untoward motion. Furthermore, we have shown that endurance training results in an up-regulation of alpha-2 adrenergic receptors, and others have demonstrated that aerobic exercise decreases tolerance to untoward motion (16,17,19). Since other polymorphic mutations may affect expression of this gene, and therefore phenotypic expression, we have begun sequencing the coding and flanking regions of this gene from many of our subjects.

First, to determine the location of the C1 alpha-2

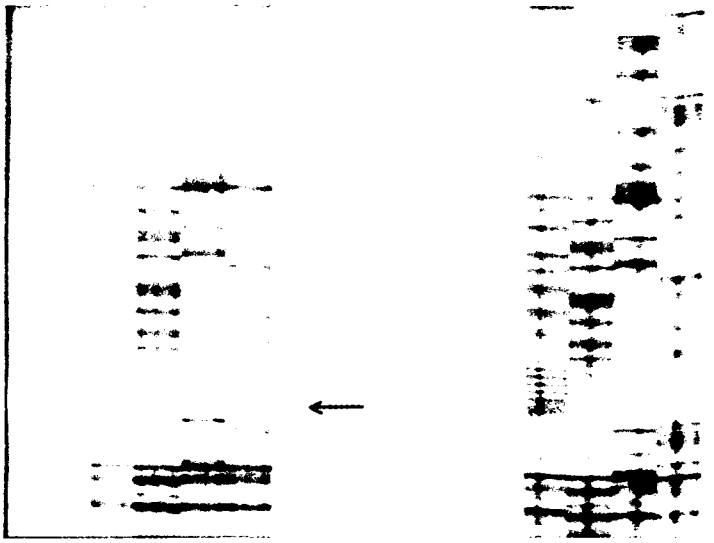


Figure 8. Sequence from cloned 6.7 (left) and 6.3 (right) homozygote gene. Lanes read T-C-G-A. Arrow denotes a G insertion in the 6.7 polymorphism.

adrenergic receptor polymorphic site identified by Dra I, we digested 10 μg of genomic DNA from a 6.7/6.3 heterozygote with both Bam HI and Dra I. Hybridization of this double-digest genomic DNA with a 5.5 kB Bam HI genomic DNA fragment yielded bands of 3.9 and 4.3 kB. This placed the polymorphic site between Hind III sites downstream of the coding sequence on the 5.5 kB Bam HI genomic DNA fragment used as a probe. Isolation of a 2.3 kB Hind III/Bam HI fragment and subsequent digestion with Dra I yielded a 1.3 kB fragment and confirmed a Dra I site approximately 800 bp downstream of the coding sequence between two Hind III sites. To determine the identity of this polymorphism, we isolated the 1.3 kB Hind III fragment from the original 5.5 kB Bam HI genomic DNA clone, ligated and cloned the fragment into a p-Bluescript SK (+) vector, and we sequenced the region of this gene in both directions using M13 and T7 universal primers. The 6.7 kB allele had a single "G" insertion in the corresponding Dra I site of the 6.3 kB clone (Figure 8). These studies await transfection assays to determine whether this mutation indeed affects message expression.

SUMMARY

Most likely, a predisposition to motion sickness is inherited as a complex, quantitative trait. Such traits are thought to have a genetic factor, but that component is not yet known. There is not only variation between individuals in the type of different stimuli which provoke motion sickness, but there is also variation in the severity in which humans respond adversely to identical provocative stimuli. It is suggested from preliminary findings in our laboratory that a genetic polymorphism of the alpha-2 adrenergic receptor encoded by chromosome 10 is associated with the development of motion sickness in man. Genetic differences in the complement of other receptors on autonomic neurons of the central and peripheral nervous system could explain human differences in susceptibility to motion sickness.

We are now using sib-pair analysis, anonymous markers, and reverse genetics to find other genes associated with a predisposition for motion sickness. With these techniques, it is possible to identify the gene(s) associated with a certain trait although the product of the particular gene is not known. By subsequently characterizing the product of that gene, other physiologic mechanisms responsible for a heritable predisposition for motion sickness can be identified. Such an approach can also be used to understand the etiology and variation in human responses to other physical stresses in the operational environment, such as a predisposition to heat stroke or gravity-induced loss of consciousness. Our goal is not to identify

individuals predisposed to motion sickness. Instead, by better understanding the cellular and molecular physiology of gene expression, more effective and logical countermeasures can be developed. After all, as any individual who has been through Navy aviator or BUD/s training knows, learning and training can overcome most genetic disadvantages.

ACKNOWLEDGEMENTS

A special thanks is extended to many of the volunteers from the Naval Special Warfare Center, BUD/s, SEAL Team 3, and the students of the University of Michigan for their enthusiasm and participation in these studies.

REFERENCES

1. Crowley JS. Simulator sickness: A problem for Army aviation. Aviat Space Environ Med 1987;58:355-57

2. Geeze DS, Pierson WP. Air sickness in B-52 crew members. Military Med 1986;151:628-29

3. Strongin TS, Charlton SG. Motion sickness in operational bomber crews. Aviat Space Environ Med 1991;62(1):57-59

4. Braithwaite MG, Braithwaite BD. Simulator sickness in an Army simulator. J Soc Occupational Med 1990;40(3):105-10

5. Johanson DC, Terry TM. An in-depth look at the incidence of in-flight loss of consciousness with the US naval service: A final report. NWC TP 6737, Naval Weapons Center, China Lake, CA, 1986

6. Kennedy RS, Fowlkes JE, Berbaum KS, Lilienthal MG. Use of a motion sickness history questionnaire for prediction of simulator sickness. Aviat Space Environ Med 1992;63(7):588-93

7. Norfleet WT, Peterson RE, Hamilton RW, Olstad CS. Susceptibility of divers in open water to motion sickness. Undersea Biomed Res 1992;19(1):41-47

8. Oman CM. Motion sickness: A synthesis and evaluation of the sensory conflict theory. Can J Physiol Pharmacol 1988;68:294-303

9. Cheung BS, Money KE, Kohn RL, Kinter LB. Investigation of anti-motion sickness drugs in the squirrel monkey. J Clin Pharmacol 1992;32(2):163-75

10. Cornelium CE, Freddland RA. Fasting hyperbilirubinemia in normal squirrel monkeys. Lab Animal Sci 1992;42(1):35-37

11. Hu S, Stern RM, Koch KL. Electrical acustimulation relieves vection-induced motion sickness. Gastroenterology 1992;102(6):1854-58

12. Lockette W, Shepard N, Lyos A, Boisemer T, Mers A. Altered Coriolis stress susceptibility in essential hypertension. Am J Hypertension 1991;4:645-50

13. Graybiel A, Woods C, Miller E, et al. Diagnostic criteria for grading the severity of acute motion sickness. Aerospace Med 1968:453-56

14. Miller EF, Graybiel A. A provocative test for grading susceptibility to motion sickness yielding a single numerical score. Acta Otolaryngologica 1970;Suppl 274:1-22

15. Kurtz T, Spence MA. Genetics of essential hypertension. Am J Med, in press

16. Lockette W, McCurdy R, Smith S. Endurance training and human alpha-2 adrenergic receptors on platelets. Med Sci Sports Exer 1987;19:7-10

17. Farrow S, Lockette W, et al. Effect of the alpha-2 adrenergic antagonist yohimbine on orthostatic tolerance. Hypertension 1990;15:877-80

18. Kohl RL, Calkins D, Mandell A. Arousal and stability: The effects of five new sympathomimetic drugs suggest a new principle for the prevention of space motion sickness. Aviat Space Environm Med 1986;57:137-43

19. Cheung BS, Money KE, Jacobs I. Motion sickness susceptibility and aerobic fitness: A longitudinal study. Aviat Space Environ Med 1990;61(3):201-204

20. Lockette W, Ghosh S, Farrow S, et al. Genetic polymorphism of the alpha-2 adrenergic receptor in Blacks with essential hypertension. Am J Hypertension, submitted

21. Eversmann T, Gottsman M, Uhlich E. Increased secretion of growth hormone, prolactin, antidiuretic hormone, and cortisol induced by the stress of motion sickness. Aviat Space Environ Med 2978;49:53-57

22. Koch KL, Summy-Long J, Bingham S, et al. Vasopressin and oxytocin responses to illusory self-motion and nausea in man. J Clin Endo Metab 1990;71(5):1269-75

23. Fibiger HC, Damsma G, Day JC. Behavioral pharmacology and biochemistry of central cholinergic neurotransmission. Adv Exp Med Biol 1991;295:399-414

24. Packard MG, Regenold W, Quirion R, White NM. Post-training injection of the acetylcholine M2 receptor antagonist AF-DX 116 improves memory. Brain Res 1990;524(1):72-76

25. Haroutunia V, Kanof PD, Tsuboyama G, Davis KL. Restoration of cholinomimetic activity by clonidine in cholinergic plus noradrenergic lesioned rats. Brain Res 1990;507(2):261-6

26. Kobilka BK, Matsui H, Kobilka TS, et al. Cloning, sequencing, and expression of the gene coding for the human platelet alpha-2 adrenergic receptor. Science 1987;247:650-56

27. Hoehe MR, Berrettini WH, Lentes KU. Dra I identifies a two allele DNA polymorphism in the human alpha-2 adrenergic receptor gene (ADAR) using a 5.5 kB probe (p ADRAR). Nucleic Acids Res 1988;16(18):9070

28. Jackson RJ, Standart N. Do the poly(a) tail and 3' untranslated region control mRNA translation? Cell 1990;62:15-24

29. Miles P, Tranchida P, MacKenzie S, Lockette W. Comparison of single- and multi-locus gene probes in DNA fingerprinting. Aviat Space Environ Med, submitted

Project Summaries FY92

Title **Characterization of Surface Antigens of Treponema Denticola**

PI Angela M. Nilius, Ph.D.
Naval Dental Research Institute
Building 1-H
Great Lakes, IL 60088-5259

Objective

Periodontal infections are a serious problem for Navy and Marine Corps mission effectiveness due to loss of personnel to dental crises and emergency treatments. Periodontal diseases are a series of inflammatory diseases of the periodontal tissues, with subsequent destruction of the alveolar bone and soft tissue support of affected teeth. The result frequently is tooth loss. The primary causes of adult periodontitis are chronic infection of periodontal tissues by a complex variety of oral bacteria (over 85 species) and the host response to the infection. One of the most important known bacterial agents of periodontitis is the bacterium, Treponema denticola.

The objective of this study is to characterize the antigens on the surface of the T. denticola bacterium (surface molecules can modulate host responses during infection and may be directly harmful to the host). Grouping strains by shared and unique antigens will have diagnostic, epidemiological, and pathological significance. The identification of genus-specific, species-specific, and serovar-specific antigens will be applied to the development of monoclonal and polyclonal antibodies, DNA probes, and biochemical tests for the rapid diagnosis of periodontal diseases associated with T. denticola.

Approach

Surface molecules are isolated from the outer membrane of T. denticola strains representing previously identified serovars as well as other oral treponemes (T. vincentii, T. phagedinis, and others). Antigens are identified using electrophoresis, Western blot, and enzyme assay techniques; animal antisera; murine monoclonal antibodies; and human salivary, tissue, and serum antibodies. The antigenic profiles of the various T. denticola strains are compared in order to identify genus-specific, species-specific, serovar-specific, and unique surface molecules. Selected antigens are purified and characterized biochemically.

Results

Surface molecules from outer membrane extracts of 11 strains of T. denticola were separated by gel electrophoresis and reacted with silver stain or antibodies. Protein profiles after silver staining were heterogeneous; however, Western blot analysis using antibody binding demonstrated two common antigens in all bacterial strains tested (molecular weights of 35.9 kDa and 32.5 kDa) and a 14.8 kDa serovar b-specific antigen in the three serovar b isolates. These results indicate that T. denticola strains share common and serovar- specific antigenic determinants which may be useful in developing diagnostic tests for periodontal disease. Additionally, it was determined that lipooligosaccharide antigens are responsible for the serovar-specificity of T. denticola (this is the first report identifying lipooligosaccharide in T. denticola and demonstrating its importance in the serovar specificity of whole cells).

Periplasmic flagella were purified from five treponemal species. All flagella contained five polypeptides with similar molecular weights and the 33 kDa common spirochete flagellar antigen. Flagellar of all T. denticola strains carried three antigenically similar polypeptides which were detected with antiserum to either whole cells of T. denticola or purified T. denticola flagella. These results confirmed the immunological conservation of flagella antigens in oral spirochetes.

Fiscal Data ($K)

FY90	FY91	FY92	TOTAL
50.0	58.2	36.0	144.2

Presentations and Abstracts

Nilius, A., and L. Simonson. 1992. Identification of two common surface antigens in Treponema denticola strains. General Meeting of the American Society for Microbiology, New Orleans, LA.

Nilius, A. M., and L. G. Simonson. 1990. Lipooligosaccharide antigens determine Treponema denticola serotype. Annual Meeting of the American Society for Microbiology, Anaheim, CA.

Title

The Role of Lymphokines in the Generation and Maintenance of Enteric Immune Responses in Mice

PI

Florence M. Rollwagen, Ph.D.

Infectious Diseases Department
Naval Medical Research Institute
Bethesda, MD 20889-5607

Objective

Campylobacter bacteria are an important cause of diarrheal disease in military personnel throughout the world. The protection of troops from these enteric pathogens will require a better understanding of both the bacteria's virulence factors and the host's responses to campylobacter invasion. One aspect of the host immune response that is presently poorly understood is the role of various lymphokines (cytokines), naturally occurring chemicals that are known to be critical intermediates in the mechanisms of disease and the development of immunity.

The objective of this project is to determine the effects of cytokines on intestinal colonization of Campylobacter jejuni in mice and their role in the generation and maintenance of the immune response to campylobacter. To facilitate this analysis, new models of cytokine administration are investigated.

Approach

The approach is twofold: 1) to explore the effects of oral administration of cytokines (interleukin-(IL)-4, IL-5, and IL-6) in augmenting in vivo enteric immune responses, and 2) to investigate the dermal administration of cytokines in agarose, to determine the in vivo chemoattraction and functions of cytokine- activated cells.

Results

Two in vivo models for the local delivery of cytokines were developed. The first model uses blocks of agarose infused with cytokines and implanted in specific tissues. Recovering the agarose blocks allows study of the kinetics and phenotypes of the immune cells attracted by the cytokines and entering the blocks, as well as the types and functions of immune cells harvested from the blocks and subsequently cultured in vitro. This cytokine-agarose model provides a unique capability for studying cytokine control of the local immune responses that important for the control of microbial infections.

The second model allows the delivery of cytokines to the intestinal mucosa and the study of cytokine effects on the mucosal immune and inflammatory responses to C. jejuni infection. Mice were fed recombinant human cytokines before and after infection with the bacteria. Intestinal lavage fluid and serum were collected and assayed for immunoglobulin and cytokines. Effects on C. jejuni colonization of the gut were determined by analyzing the number of colony-forming units per milligram of mouse feces.

It was found that antigen-specific humoral immune responses were developed in response to C. jejuni infection in mice. Cytokines were detected in lavage fluid following infection. IL-l and IL-6 were found, but not IL-2. Serum cytokine levels revealed the presence of early intermittent low levels of IL-1 and IL-6. IL-2 levels were markedly elevated and sustained. Administration of IL-6 resulted in an augmentation of host defenses which led to early elimination of bacteria. IL-5 or IL-2 administration induced a delayed effect on the course of infection.

Fiscal Data ($K)

FY90	FY91	FY92	Total
95.0	87.2	90.6	272.8

Publications

Rollwagen, F. M., N. D. Pacheco, and S. Baqar. 1992. An improved model for the examination of biological effects of locally administered cytokines. J. Immun. Meth. (in press)

Rollwagen, F. M., N. D. Pacheco, and S. Baqar. 1992. An improved model for the examination of biological effects of locally administered cytokines. NMRI Technical Report.

Presentations and Abstracts

Rollwagen, F. M., S. Baqar, N. D. Pacheco, B. Spargo, and A. Rudolph. 1992. Administration of cytokines in agarose attracts different cell populations to the local site. Annual Meeting of the Federation of American Societies for Experimental Biology, Anaheim, CA.

Baqar, S., N. D. Pacheco, and F. M. Rollwagen. 1992. Mucosal and systemic immune responses to Campylobacter jejuni in mice. Annual Meeting of the Federation of American Societies for Experimental Biology, Anaheim, CA.

Rollwagen, F. M. 1992. Administration of cytokines in agarose attracts different cell populations to the local site. Minisymposium "Cytokines in Tissues". Annual Meeting of the Federation of American Societies for Experimental Biology, Anaheim, CA.

Baqar, S. 1992. Mucosal and systemic immune responses to Campylobacter jejuni in mice. Minisymposium "Cytokines in Tissues". Annual Meeting of the Federation of American Societies for Experimental Biology, Anaheim, CA.

Rollwagen, F. M. 1992. Cell mediated reactions: effector molecules and effector mechanisms. University of Maryland, Baltimore, MD.

Baqar, S. 1992. Role of cytokines in Campylobacter jejuni infection and immunity in mice. Ph.D. Thesis. University of Maryland, Baltimore, MD.

Title **Studies on Purified Cord Blood Hematopoietic Cells**

PI Steven Kessler, Ph.D.
Immune Cell Biology Program
Naval Medical Research Institute
Bethesda, MD 20889-5607

Objective

Personnel exposed to bone marrow ablative agents during military conflicts will likely require bone marrow transplantation for survival. As currently practiced, bone marrow transplantation is subject to two major limitations. First is the need to recruit for initial tissue typing and maintain a registry of potential volunteer donors. Second is the need to test large numbers of potential donors (if a related donor is unavailable) to find an identical tissue histocompatibility match (estimated at 1 in over 20,000), in order to avoid graft-versus-host disease (GVHD). Advanced therapies for treating bone marrow failure clearly are needed to resolve these limitations.

This project focused on evaluating a potential second source of hematopoietic stem cells for reconstituting injured or destroyed bone marrow. It was hypothesized that such stem cells might be isolated from cord blood (vice bone marrow) in numbers sufficient to reconstitute bone marrow in both autologous and allogeneic transplantation. The elimination of mature T lymphocytes (capable of initiating GVHD) through stem cell purification would extend the feasibility of using this technique for allogeneic transplantation to unrelated recipients. Also, stem cells may serve as "universal donors" by giving rise to lymphoid progeny that are tolerant to the recipient or host. Combined with the use of currently available regimens for immunosuppression or growth factor administration to facilitate engraftment, this technique may reduce the need for stringent tissue type matching of donors and recipients.

Approach

Stem cells were obtained from umbilical cord and placental tissue blood scheduled for discard at the Department of Obstetrics and Gynecology, Naval Hospital Bethesda. Blood was freed of red blood cells and granulocytes; CD34 + stem cells then were positively selected using a monoclonal antibody to the CD34 antigen. The cells were characterized phenotypically and functionally, in comparison with counterparts isolated from bone marrow. Cells were quantified in short-term clonogenic assays and in long-term culture systems before and after cryopreservation.

Results

Results show that the techniques developed for bone marrow-derived stem cells are applicable to cord blood. The number of stem cells recovered from umbilical cord blood under optimized conditions proved to exceed typical recoveries from aspirated bone marrows by 5- to 10-fold. Additionally, a cell line of normal human thymic stromal cells was developed, which may support the long-term culture of stem cells and reduce the dependence on primary bone marrow.

Fiscal Data ($K)

FY91	FY92	TOTAL
90.3	80.6	170.9

Title

The Role of Neuropeptide Y on the Acquisition and Performance of Response Sequences During Heat Stress

PI

John Schrot, Ph.D.

Thermal Stress Program
Naval Medical Research Institute
Bethesda, MD 20889-5607

Objective

Thermal stress is often a major element in Navy and Marine Corps operational environments. Exposure to stressful situations releases neuropeptides in the brain that have profound effects on cognitive abilities. The basic mechanisms of how stress-induced neuropeptide release affects learning and memory have not been well defined. Neuropeptide Y (NPY) coexists with stress hormones in areas of the brain essential for normal learning. The objective this project is to examine the role of NPY as a mechanism underlying thermal stress-induced effects on cognitive functioning. Information generated by this research may contribute to the development of pharmacological strategies to protect personnel from the deleterious effects of stress.

Approach

Rats are trained on a behavioral procedure (repeated acquisition) which measures learning and memory performance. NPY is administered to these animals, intracerebroventricularly through indwelling catheters, at various times in the learning process, and under varying thermal conditions. Measuring rat performance on learned tasks will provide information about both the effects of NPY on learning in progress and on well learned behavior. The mechanisms of action of NPY can later be determined by administering either NPY antagonists or fragments into several sites within the hippocampus and amygdala, brain regions known to be important for normal learning.

Results

During the first months of this project, training programs were written in the MED PC language and animals were trained on the repeated acquisition procedure. In this training, each animal participates in a series of sessions in which it learns a sequence of responses in order to obtain a food reward. Three response levers (R,C,L) must be pressed in the correct order for food to be delivered (for example, during one session, the correct sequence of responses might be CLR, and on the following session it might be RLC). Each session consists of 150 lever sequences and usually takes between 60 and 90 minutes to complete. This procedure allows generating daily learning curves, providing baselines against which the effects of heat and NPY can be measured.

Initial experiments have focused on defining the magnitude of heat effects (31°C, 35°C, and 38°C) on rat behavioral performance. Acute exposure to heat produced a decrease in the accuracy of responding on both the acquisition and performance baselines. Error responding increased during both conditions when air temperatures were either 35°C, or 38°C. Exposure to 31°C air produced occasional sessions of increased response errors.

Fiscal Data ($K)

FY92	FY93	TOTAL
70.1	59.8	129.9

Presentations and Abstracts

Schrot, J. and J. R. Thomas. 1992. Exposure to heat stress disrupts the acquisition and performance of response chains in rats. Annual Meeting of the Society for Neuroscience, Anaheim, CA.

Title **Idiotypic Mimicry of Endotoxin and Endotoxin Receptors**

PI Che-Hung R. Lee, Ph.D.
Septic Shock Research Program
Naval Medical Research Institute
Bethesda, MD 20889-5607

Objective Gram-negative (G(-)) sepsis and septic shock are major complications and constant threats following combat injury. Endotoxin (or lipopolysaccharide, LPS), ubiquitously existing on the cell wall of G(-) bacteria, has been identified as one of the major causes of this disorder. Lipid A, the most conserved portion of LPS structure, has proved to be the toxic portion of endotoxin. The objective of this study is to examine the mimicry of the structures and functions of endotoxin and endotoxin receptors by antiidiotypic monoclonal antibodies (AIMAbs) and monoclonal antibodies (Mabs) against lipid A. These molecules might be used to neutralize lipid A and LPS function, induce cytokines to protect animals from septic shock, and to prevent lipid A and LPS from binding to LPS receptors and initiating the frequently lethal septic shock cascade.

Approach The specific and non-specific binding sites of LPS on endothelial cells are determined using ^{125}I-labeled LPS. AIMAbs are used to compete against LPS for binding to the cells, illustrated by fluorescence and electron microscopies. The mimicking of the LPS function by the AIMAbs is determined by their ability to inhibit or enhance endothelial cell detachment caused by LPS (monitored by ^{3}H-thymidine incorporation and trypan blue exclusion). AIMAbs and LPS are used to induce endothelial cells to produce cytokines, interleukin (IL)-I, IL-6 and tumor necrosis factor (TNF).

Results Radioimmunoassays for detecting expression of the surface adhesion molecules, ICAM-1 and ELAM-1, on human umbilical cord vein endothelial cells were developed and the endotoxin-induced expression of ICAM-1 in these cells was studied. It was shown that LPS as low as 1 ng/ml induces ICAM-1 expression and that expression is maximal after exposure to 1 /ml LPS for 48 hrs. The next step in this study will be to determine how Mabs and AIMAbs can inhibit or induce the expression of these adhesion proteins. Using immunofluorescence staining, AIMAbs were shown to bind to cultured human umbilical cord vein endothelial cells with a patchy distribution. In rat peritoneal macrophage cultures, AIMAbs showed variable ability to stimulate these cells to produce IL-1, IL-6, and/or TNF. Competitive inhibition enzyme-linked immunosorbent assays were used to characterize the antiidiotypes of the AIMAbs. AIMAb binding was specific to the antibody fragments bound to the test vessel and some of this binding was inhibited by lipid A.

Fiscal data ($K)

FY92	FY93	TOTAL
98.6	100.0	198.6

Presentations and Abstracts

Lee, C. H., Y. Reid, J. S. Jong, T. Williams, and Y. H. Kang. 1992. Endotoxin-induced differential expression of ICAM-1 in human umbilical cord vein endothelial cells (HUVEC) culture. Circulatory Shock. 37:30.

Lee, C. H., T. Ngo, J. S. Jong, L. White and Y. H. Kang. 1993. Mechanisms of LPS-induced ICAM-1 and ELAM-1 expression in human umbilical cord vein endothelial cell (HUVEC) culture and the salt effects. 16th Annual Conference on Shock, Santa Fe, NM, June 13-16.

Renzi, P. M. and Lee, C. H. 1993. Anti-lipid A monoclonal antibodies (MAbs) inhibit endotoxin-induced human endothelial cell responses. 16th Annual Conference on Shock, Santa Fe, NM, June 13-16.

Title

Cytokine Modulation of Cell Surface Factors and Cytoskeleton Organization in Endothelial Cells and Monocytes/Macrophages

PI

Y. H. Kang, Ph.D.
Wound Repair Enhancement
Building 17B, Room 7A
Naval Medical Research Institute
Bethesda, MD 20889-5607

Objective

Navy personnel engaged in combat or hazardous operations often suffer from traumatic injuries and microbial infections which induce inflammatory reactions. Uncontrolled inflammatory responses may result in multiple organ failure and impaired wound healing. The main objective of this project is to elucidate the molecular and cellular mechanisms that regulate the inflammatory response, so that a therapeutic strategy for controlling inflammation can be developed.

The study focuses on the effects of various factors (cytokines, growth factors, and two known mediators of inflammatory reactions (lipopolysaccharide (LPS) and thrombin) on cell surface factors and cytoskeleton reorganization in endothelial cells, monocytes and macrophages. The effects of these factors on leukocyte attachment, emigration and endothelial leakage in the endometrial vasculature and their potential to reverse or modify these changes are being studied.

Approach

Immunoelectron microscopy, immunofluorescence, radioimmunoassay and other advanced techniques are used in a rat endometrium model to study factor effects on cell surface components (receptors, cell adhesion molecules, and plasma membrane-associated enzymes on endothelial cells, monocytes and macrophages). Actin filament reorganization is evaluated by immuno-fluorescence and confocal microscopy and analyzed using an image analysis system. Cultures of human umbilical cord vein endothelial cells, human peripheral blood monocytes and synovial macrophages are used in in vitro studies on cell surface factors and cell-to-cell adhesion and interaction. Monocyte-endothelial cell adhesion is assessed using enzyme immunoassay.

Results

In vitro studies of the effects of LPS and thrombin on the expression of the fibronectin receptor (FNR), CD14 and CD18 on human monocytes and macrophages showed that LPS promotes FNR expression on monocytes, but suppresses expression on macrophages, in a dose-related fashion. Electron microscopy revealed that FNR is concentrated on the cell surface of the pseudopodia regions of monocytes. FNR was shown to be involved in LPS-mediated adhesion and migration and results suggested that LPS may inhibit macrophage migration. The effect of thrombin on FNR expression was irregular.

Initial results showed that LPS supresses both CD14 and CD18 receptors on monocytes and macrophages, in a dose-related manner. This supression may depend on prolonged incubation of the cells with LPS and a kinetic study is planned to clarify the role of FNR, CD14 and CD18 in monocyte adhesion and migration.

Fiscal Data ($K)

FY92	FY93	TOTAL
70.0	86.0	156.0

Presentations and Abstracts

Kang, Y.H., S. E. Quella, and C. H. Lee. 1993. Thrombin regulation of ICAM-1 (CD54) expression by human umbilical cord vein endothelial cells (HUVEC). 16th Annual Conference on Shock, Santa Fe, NM. June 13-16.

Kang, Y. H., S. E. Quella, R. Williams, and C. H. Lee. 1992. Thrombin modulation of expression of matrix proteins and receptors in human pulmonary artery endothelial cells. Mol. Biol. Cell. 3: 131a.

Kang, Y. H., C. H. Lee, S. E. Quella, R. Williams, and H. Newball. 1992. Endotoxin modulates expression of fibronectin and fibronectin receptor in human lung macrophages. J. Leuk. Biol., supp. 3: 47.

Kang, Y.H., C.H. Lee, R. L. Monroy and R. Williams. 1991. Bacterial endotoxin promotes expression of fibronectin receptors in human monocytes. J. Cell Biol. 115: 108a.

Title

Determination of the Mechanism(s) of Endothelial Injury During Bone Marrow Transplantation

PI

CAPT R. A. Cahill, MC, USN
Bone Marrow Registry Program
Naval Medical Research Institute
Bethesda, MD 20889-5607

Objective

Patients undergoing bone marrow transplants can suffer a number of complications post transplantation, including pneumonia, severe liver injury, infection, and graft-versus-host disease (GVHD). An additional complication that has been recognized just recently is capillary leak syndrome (CLS), characterized by excessive weight gain and non-cardiogenic edema (fluid in the lungs which can cause potentially fatal respiratory failure). CLS is associated with GVHD but occurs more often and is more serious in the unmatched transplant patient. Although the cause(s) and time course of CLS are not known, injury to cells of the microvascular endothelium appears to be an important factor.

The objective of this project is to determine the mechanism(s) of CLS-related endothelial cell damage in bone marrow transplant patients so that strategies for the prevention of this life threatening complication can be developed. This work directly supports the Navy's unrelated marrow donor program for the treatment of combat casualties with bone marrow failure.

Approach

During the preparative regimen for bone marrow transplantation, mediators from host inflammatory cells and the endothelium are believed to be released continually. These mediators initiate the adhesion and activation of donor or autologous leukocytes. In this study, putative proinflammatory mediators are measured before and after transplantation: cytokines, interleukin-1, interleukin-6, tumor necrosis factor, gamma-interferon, the arachidonic acid metabolites, thromboxame B2, and leukotrine E4. Changes in the endothelium of biopsy specimens of skin, liver and lung are observed by electron microscopy. Also, the types of cells (polymorphonuclear leukocytes, lymphocytes, monocytes, macrophages, and platelets) present in the microvascular tissue of biopsy specimens and their specific receptors/ligands (ICAM-1, LFA-1, ELAM-1, PECAM) are determined using immunocytochemistry.

Results

Levels of thromboxane B2 and leukotriene E4 were elevated in the urine of the thirty three bone marrow transplant patients who had serious manifestations of endothelial injury (fluid retention, weight gain, edema, and multiorgan failure). Both these arachidonic acid metabolites have been shown to cause CLS in experimental animals. Patients with CLS showed evidence of T-cell activation, Interleukin-8 release and Interleukin-6 release following the initial release of the arachidonic acid metabolites and occurring over a period of 2-5 days before there are any major signs of endothelial damage. Tumor necrosis factor, which primes many cells to release cytokines, was not elevated in the serum of any of the patients. The release of proinflammatory cytokines may be responsible for the migration of leukocytes and additional tissue injury during the syndrome. Ten patients have undergone skin biopsies for electron microscopy prior to the onset of the skin rash characteristic of endothelial injury. Studies revealed endothelial injury manifested by the presence of red cells and leukocytes in the extravascular spaces and disruption of the endothelium of the arterioles or larger blood vessels.

Fiscal Data ($K)

FY92	TOTAL
58.0	58.0

Title

The Use of LEET and Bright Light Separately and Together for Shifting the Work/Rest Cycle

PI

Tamsin Lisa Kelly, M.D.

Cognitive Performance and Psychophysiology Department
Naval Health Research Center
San Diego, CA 92186-5122

Objective

Navy and Marine Corps personnel often work under unusual schedules or after shifting many time zones. Sensitive operations could be compromised because of performance deterioration related to impaired sleep or having to work during the low period of the body's circadian rhythms. Techniques which facilitate rapid adaptation to a new work shift or a new time zone would be valuable in promoting mission accomplishment and the safety of deployed personnel. Pharmacological sleep aids are problematic in military environments because of the immediate and delayed detrimental effects on performance and the possibility of dependence. A non-pharmacological intervention is preferable for military use.

The objective of this study is to investigate the effectiveness of exposure to timed bright light and low amplitude electromagnetic fields (low energy emission therapy, LEET), singly or in combination, to decrease fatigue and performance deterioration due to moving from the day shift to the night shift, or to traveling across multiple time zones.

Approach

Subjects undergo a 10-hour delay in the sleep/wake cycle to simulate a transfer from the day shift to the night shift, or crossing 10 time zones. Subjects receive four hours of either bright light or dim light exposure and either actual or sham LEET treatment for three days after making this shift. Performance is assessed during the new work period using a computer-administered Performance Assessment Battery (PAB) of cognitive tasks known to be sensitive to sleep deprivation and to show variation across the circadian cycle. Sleep is recorded and analyzed before, during, and after the treatment period. Subjects are isolated from outside light during the course of the study.

Results

Polygraphs to be used for sleep recordings, LEET devices, and computers required for performance testing were established in the isolation facility and the best electromagnetic wavelength mixture to be used in these experiments was determined. The PAB was modified to contain only those performance tasks most pertinent to this study. The PAB now consists of the following tasks: addition, fourchoice reaction time, logical reasoning, tapping (lapses), single and choice reaction time, word memory, synthetic work, and visual analog sleepiness scale. All software has been tested in pilot subjects. Eight subjects have completed the protocol: 6 subjects were exposed to bright light-placebo LEET and 2 subjects received dim light-placebo LEET. Data analysis, along with actual LEET exposure experiments, is continuing.

Fiscal Data ($K)

FY92	FY93	TOTAL
69.1	106.0	175.1

Title

Plasma Volume, Vasopressin, and the Genetics of Motion Sickness

PI

Warren Lockette, M.D.
Physiological Performance and Operational Medicine Department
Clinical Investigative Medicine Division
Naval Health Research Center
P O Box 85122
San Diego, CA 92186-5122

Objective

Future conflicts involving American military forces are most likely to occur in hot weather regions, such as the Middle East. High climatic temperatures in these areas threaten aircrews, shipboard personnel and ground troops with dehydration, which commonly causes a reduction in plasma volume. It is widely recognized that decreased plasma volume (and hence, blood pressure) leads to discomfort and to reduced abilities in performing physical tasks. It is not currently known, however, whether changes in plasma volume also impact the vestibular centers controlling balance and orientation, or whether these changes exacerbate the effects other operational stressors, such as the high gravitational forces experienced in modern fighter aircraft.

The objective of this study is to determine whether decreased plasma volume contributes to vestibular dysfunction and leads to disorientation and motion sickness in susceptible personnel. A synthetic hormone (1-desamino-8-D-arginine vasopressin (DDAVP)) is being used to expand plasma volume in hopes of decreasing personnel susceptibility to disorientation and motion sickness. If it is determined that susceptibility to motion sickness is genetically controlled, the gene(s) responsible for the increased predilection of some individuals to motion sickness will be identified.

Approach

Vestibular function, postural control, Coriolis stress susceptibility, plasma volumes and levels of vasopressin and of hormones that react to physiologic stress (catecholamines, cortisol, growth hormone, aldosterone) are being measured in individuals with and without a history of motion sickness. The effect of plasma volume expansion with DDAVP on Coriolis stress susceptibility (measured as the Coriolis Stress Susceptibility Index (CSSI)) is tested in these subjects. Family studies will determine whether the trait for motion sickness is inherited in a Mendelian manner. DNA markers for motion sickness susceptibility are being identified and analyzed by Southern blot and polymerase chain reaction combined with single-stranded conformational polymorphism analysis. Computerized programs are being used to determine the likelihood of linkage between DNA markers and the locus determining the Coriolis stress phenotype.

Results

The investigators have demonstrated that DDAVP does not induce motion sickness in individuals subjected to Coriolis stress; thus, DDAVP continues to be a promising treatment for military personnel performing motioninvolving tasks in hot operational environments. A possible genetic basis for motion sickness susceptibility has been determined. Analysis of DNA isolated from 45 volunteers with normal vestibular function and their scores showed that variation in the gene coding for the alpha-2 adrenergic receptor (A2AR) may relate to susceptibility to motion sickness. Individuals heterozygous for the 6.7/6.3 alleles of the A2AR gene had significantly lower CSSI scores than did individuals homozygotic for the 6.7 allele. To facilitate further genotype analyses using polymerase chain reaction technology, the A2AR gene, including 1200 bases that encompass the region responsible for the gene's polymorphism, was sequenced. The mutation responsible for the 6.3 and 6.7 alleles was mapped to the 3' noncoding portion of the chromosome 10 alpha-2 adrenergic receptor. This genetic variation, possibly along with others yet to be identified, may explain the variety of individual responses to untoward motion.

Fiscal Data ($K)

FY92	FY93	TOTAL
67.0	115.0	182.0

Presentations and Abstracts

Lockette, W., N. Shepard, T. Boismier, S. MacKenzie, and P. Miles. 1992. Motion sickness is associated with a genetic polymorphism of the alpha-2 adrenergic receptor. Annual Meeting of the Aerospace Medical Association, Miami, FL.

Hodder, G., N. Shepard, T. Boismier, Y. Wang, S. Farrow and, W. Lockette. 1992. Vasopressin does not increase susceptibility to Coriolis stress. Annual Meeting of the Aerospace Medical Association, Miami, FL.

FY92 INDEPENDENT RESEARCH PROJECTS DATABASE

SP #	SE #	ACCESS #	PROJ #	PROJECT TITLE	PI	FUNDING $K FY90	FY91	FY92	FY93	APP 1	APP 2	DODCT
00101	41	DN240519	0062	SURFACE ANTIGENS OF TREPONEMA DENTICOLA	NILIUS	50.0	58.2	36.0	-	PMD	FSO	BIOTEC
00101	41	DN240526	1384	LYMPHOKINES/ENTERIC IMMUNE RESPONSE	ROLLWAGEN	95.0	87.2	90.6	-	PMD	FSO	
00101	41	DN241505	1392	CORD BLOOD STEM CELLS	KESSLER	-	90.3	80.6	-	PMD	FSO	
00101	41	DN242601	1396	NEUROPEPTIDE Y AND PERFORMANCE IN HEAT	SCHROT	-	-	70.1	59.8	PMD	FSO	
00101	41	DN242602	1397	IDIOTYPIC MIMICRY OF ENDOTOXIN RECEPTORS	LEE	-	-	98.6	100.0	PMD	FSO	BIOTEC
00101	41	DN242622	1398	CYTOKINE MODULATION OF ENDOTHELIAL CELLS	KANG	-	-	70.0	86.0	PMD	FSO	
00101	41	DN242623	1399	ENDOTHELIAL INJURY IN TRANSPLANTATION	CAHILL	-	-	58.0	-	PMD	FSO	
00101	41	DN242624	6044	LEET/BRIGHT LIGHT IN WORK/REST CYCLES	KELLY	-	-	69.1	106.0	PMD	FSO	
00101	41	DN242625	6045	PLASMA VOLUME/VASOPRESSIN/ MOTION SICKNESS	LOCKETTE	-	-	67.0	115.0	PMD	FSO	BIOTEC
							TOTAL	640.0 [a]				

[a] In FY92 all (100%) IR funds were spent on the salaries and technical support of in-house principal investigators.

Baqar, S. 1992. Mucosal and systemic immune responses to Campylobacter jejuni in mice. Minisymposium "Cytokines in Tissues." Annual Meeting of the Federation of American Societies for Experimental Biology, Anaheim, CA.

Rollwagen, F. M. 1992. Cell mediated reactions: effector molecules and effector mechanisms. University of Maryland, Baltimore, MD.

Baqar, S. 1992. The role of cytokines in infection and disease. University of Maryland, Baltimore, MD.

Spargo, B. J., A. S. Rudolph, and F. M. Rollwagen. 1993. Temporal and spatial recruitment of tissue resident cells to hydrogel composites in response to lipid microcylinders and cytokines. 19th Annual Meeting of the Society for Biomaterials, Birmingham, AL.

Spargo, B. J., G. E. Stilwell, R. O. Cliff, R. L. Monroy, F. M. Rollwagen, and A. S. Rudolph. 1991. Technological development of lipid-based microcylinders: biocompatibility and controlled release. Material Research Society Symposium on Tissue Inducing Biomaterials.

Spargo, B. J., A. S. Rudolph, and F. M. Rollwagen. 1993. Recruitment of tissue resident cells to hydrogen composites: in vivo response to implant materials. 4th International Symposium of Biointeractions. Molecular aspects of biomaterials, Noordwijkerhout, Netherlands.

NMRDC is very proud of the accomplishments of Florence Rollwagen, Nancy Pacheco, and Shahida Baqar and is pleased to present their work as an example of the outstanding biomedical research performed for the Navy through NMRDC's IR program.